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Special Issue on:
Photo-physiology of marine organisms

Acropora muricata bleaching photo by Dr. Kristen Brown

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The usage of "et al." in long author lists will also be accepted:

Smith J, Jones M Jr, Houghton L et al. 1999. Future of health insurance. N Engl J Med 965: 325-329. DOI: 10.10007/s002149800025.

Book:

Rai MK, Carpinella C. 2006. Naturally Occurring Bioactive Compounds. Elsevier, Amsterdam.

Chapter in the book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds.). Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract:

Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta*. 50th annual symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.). Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

Information from the internet:

Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. Mol Syst Biol 4: 187. DOI: 10.1038/msb.2008.24. www.molecularsystembiology.com.

EDITORIAL NOTE
For Special Issue on

"Photo-physiology of marine organisms"

Indo-Pacific Journal of Ocean Life vol. 7, no. 1, June 2023

Greetings!

This special edition of "*Photo-physiology of marine organisms*" in the Indo-Pacific Journal of Ocean Life is dedicated to the late Dr. Ricardo F. Tapilatu from the Pacific Marine Resources Research Center, University of Papua (UNIPA), Manokwari, Indonesia, and the then Editor-in-Chief. Dr. Tapilatu received the request in August 2021, and the Editorial Office launched this special edition announcement in June 2023.

The team members worked under the Pole of Research Excellence in Sustainable Marine Biodiversity (PRE-SMB), led by Dr. R. Bhagooli and the Department of Biosciences & Ocean Studies, Faculty of Science at the University of Mauritius. They submitted several manuscripts related to photo-physiological studies of marine organisms around the waters of the Republic of Mauritius. The peer-reviewed papers are presented under the special issue entitled "*Photo-physiology of marine organisms*." This special issue is presented in the context that the waters of the Republic of Mauritius in the Indian Ocean possess one of the most biodiverse but fragile coral reefs and mangrove ecosystem-associated organisms. These ecosystems provide ecological goods and services supporting coastal and national economies. Still, the same ecosystems are now at peril after extreme climatic phenomena, including climate change-driven ocean warming.

This special edition includes the photo-physiological features and stress responses in several photosynthetic marine organisms and/or marine organisms in symbiotics with photosynthetic microalgae. The original article by Narrain et al. documented three macroalgae variables: photosynthetic, phytochemicals, and anti-oxidative responses. At the same time, Gopeechund et al. present photo-physiological and anti-oxidant acclimatization in macroalgae in different nutrient regimes. Using macroalgae could attract more attention, especially in developing the "Blue Economy" concept in developing countries. Fai et al. present photosynthetic responses and distribution of the ascidian *Diplosoma simile*. The opportunistic or invasive behavior of *D. simile* seems important to be characterized as mass coral bleaching/mortality events increase in severity and frequency worldwide. Jogee et al. present spatial photo-physiological responses of diseased and non-diseased coral parts, while Mundil et al. report the prevalence and photo-physiological thermal responses of the coral disease, Skeletal Eroding Band (SEB), caused by the ciliate *Halofolliculina corallasia*, in *Acropora muricata*.

Coral diseases are increasingly being reported worldwide as local and global stressors. They represent a lethal threat to the productivity and subsistence of coral reefs, especially in the wake of climate change. Ricot et al. investigate the photo-physiological responses of fish predated and non-predated parts of a thermally resilient coral, *Porites lutea*. It is important to test the impacts of other biological stressors, such as fish predation, on coral surviving ocean warming events to inform better coral management and conservation. Jeetun et al. investigated whether the thermal photosynthetic responses of *A. muricata* are enhanced following a coral field transplantation experiment, while Munbodhe et al. assessed the thermal tolerance of endemic/rare corals. As coral bleaching/mortality events appear more pronounced, it is essential to test whether corals can acclimatize or adapt to different thermal regimes. Ramah et al. documents variable photosynthetic thermal stress responses between two species of giant clams (Tridacnines), *Tridacna maxima* and *T. squamosa*, from Rodrigues Island. Although giant clams (Tridacinidae) are protected in many countries, the decline in their population is increasingly being reported. That is important to understand whether global warming is a major contributor must be determined for its conservation. Kaullysing et al. present a difference in photo-physiological responses of various parts of the salt-tolerant tropical mangrove plant, the *Rhizophora mucronata*. Therefore, it is worth understanding the photosynthetic contributions of various tree parts as they are subject to varying environmental conditions. Perrine et al. extend the thermal stress studies on Red Coralline Algae from Mauritius Island. The study on understanding the thermal photo-physiological susceptibility of *Hydrolithon onkodes*, an attractor of coral larvae, has important implications for managing the recovery of thermal stress-affected reefs. Finally, this special issue ends with findings presented by Soondur et al. relating to micro-phytoplankton density, diversity, and photophysiology from degraded and non-degraded reefs around Rodrigues Island.

The contributions of this special edition foster new insights into the photo-physiology of marine organisms through the application of chlorophyll fluorescence technique using the Pulse-Amplitude-Modulated fluorometer. Furthermore, the findings presented here may further inform decision-making for coral reefs and mangrove conservation and management in the Republic of Mauritius and the wider Indian Ocean.

Associate Professor (Dr.) Ranjeet Bhagooli
Editor-in-Chief

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Differential photosynthetic, phytochemical and antioxidative responses of three macroalgae *Ulva lactuca*, *Gracilaria salicornia* and *Turbinaria ornata* exposed to thermal and irradiance conditions

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Abstract. *Narrain D, Baulroop J, Bhagooli R, Bahorun T. 2023. Differential photosynthetic, phytochemical and antioxidative responses of three macroalgae* Ulva lactuca, Gracilaria salicornia *and* Turbinaria ornata *exposed to thermal and irradiance conditions. Indo Pac J Ocean Life 7: 1-15.* Worldwide climate change leads to a varied distribution of aquatic organisms due to their differences in susceptibility to environmental conditions. Being at the base of marine food webs, macroalgae are potential candidates to investigate the effects of changing environmental conditions and to study the adaptation mechanisms. This study examined the effects of in vitro thermal and irradiance conditions (Control - CLCT: 1.55±0.63 µmol quanta $m^{-2}s^{-1}$ and 28°C; Control light and high temperature - CLHT: 1.55±0.63 µmol quanta $m^{-2}s^{-1}$ and 32°C; Moderate light and control temperature – MLCT: 100±63.6 µmol quanta $m^{-2}s^{-1}$ and 28°C; Moderate light and high temperature – MLHT: 100±63.6 µmol quanta $m^{-2}s^{-1}$ and 32°C) for 1 week on the photosynthetic performance, phytochemical contents, and antioxidant potential of three macroalgae *Ulva lactuca* L., *Gracilaria salicornia* (C.Agardh) E.Y.Dawson and *Turbinaria ornata* (Turner) J.Agardh found in the lagoons of Mauritius Island. Our results indicate variable responses of the three test macroalgal species when exposed to combinations of temperature and light conditions. Differential responses were found to be both species- and stress-specific. Chlorophyll fluorescence measurements using a Diving Pulse-Amplitude Modulated (D-PAM) fluorometer indicated a significant increase ($p<0.001$) in relative maximum electron transport rate (rETRmax) of *U. lactuca* in all stress treatments implying higher photosynthetic activity compared to control conditions. A significant decrease ($p<0.001$) in rETRmax of *G. salicornia* under MLHT and the collapse of photosystem II (PSII) activity (Fv/Fm) in *T. ornata*, along with both species exhibiting visual pigment degradation, are suggestive of chronic photo-inhibition in these two macroalgal species. Antioxidant activities (FRAP and TEAC assays) correlated stronger to flavonoid contents (FRAP, r=0.909; TEAC, r=0.845) than to phenol contents (FRAP, r=0.688; TEAC, r=0.758). An increase in temperature and irradiance severely damaged the PSII of *T. ornata* and *G. salicornia*, while *U. lactuca* could photo-physiologically adjust to changing environmental conditions, showing its robustness. The elevated temperature significantly affected the photosynthetic performance and antioxidative activities of the tested macroalgal species ($p<0.001$). These findings are discussed to possible influence on defense mechanisms of these macroalgal species and their aquaculture potential in an era of climate change. Further research using field-based manipulations as well as molecular analysis is warranted to thoroughly understand the potential mechanisms involved in variable responses of these tested macroalgae.

Keywords: Antioxidant activity, climate change, marine macroalgae, photophysiology, phytochemistry, pulse-amplitude modulated fluorometry, thermal stress

INTRODUCTION

Marine macroalgae, commonly known as seaweeds, are macroscopic and multi-cellular autotrophic organisms. These aquatic organisms are ecologically, biologically, and economically important as they provide medicinal compounds (Pribadi and Kanza 2017; Kalasariya et al. 2022; Kumar et al. 2022; Naqvi et al. 2022), serve as habitats for other living organisms (Fulton et al. 2020), serve in agriculture as bio-pesticides (Asimakis et al. 2022) and bio-fertilizers, contribute in bioremediation (Voskoboinikov et al. 2021), as well as play a major role in carbon sequestration (Gao and Beardall 2022), nutrition and habitats for other living organisms. Macroalgae, the primary producers in the marine food web, macroalgae represent key components within coastal ecosystems (Setyorini et al. 2021). However, the rise in global warming- and climate change-driven environmental changes (Harley et al. 2012; Meethoo et al. 2017) are having drastic impacts on macroalgal physiology, growth, reproduction, and survival (Ji and Gao 2021). These marine autotrophs are highly affected by fluctuating environmental conditions derived from climate change and anthropogenically-driven phenomena. Elevated temperatures, intense sunlight, rapid salinity, nutrient changes, desiccation, and numerous forms of pollution are among the major stress inducers (Kakinuma et al. 2001) that make macroalgae vulnerable by influencing their performance

and functioning at molecular and physiological levels (Smolina et al. 2016), eventually impacting on their survivorship, distribution, and diversity. Therefore, these organisms give an account of the environmental conditions they can live in and the adaptations they have acquired (Ji and Gao 2021).

These photosynthetic organisms' tolerance to environmental stresses, such as temperature and light, can be assessed by their photosynthetic performance and defense mechanisms, for instance, enzymatic antioxidant system, non-enzymatic antioxidant system, and heat shock response (Harley et al. 2012; Smolina et al. 2016). Photosynthesis is a well-established source of reactive oxygen species (ROS) in autotrophs, including marine macroalgae (Carvalho et al. 2004). The photosynthetic electron transport chain occurs in the Photosystems I and II of the thylakoid membranes (Moustakas 2021). It is the principal site where ROS, such as superoxide anion radical (O2 •−), hydrogen peroxide (H2O2), and singlet oxygen (1O2), are continuously produced at basal levels as part of the normal metabolic process and are scavenged by different antioxidant mechanisms. However, when exposed to stress conditions, there is an overproduction of ROS. ROS are toxic and damage proteins, lipids, carbohydrates, and DNA, eventually leading to oxidative stress (Gill and Tuteja 2010). For instance, at low tides, macroalgae are exposed to increasing temperature, UV light, and excessive exposure to photosynthetically active radiation (PAR) (Brown 1997), all known to induce oxidative stress (Lesser 2006; Tal et al. 2011; Maharana et al. 2015). In such conditions, photoprotection mechanisms preventing ROS formation through non-photochemical quenching (NPQ) or by scavenging ROS are activated to prevent damage and improve stability. Some of the main stress-related photo-physiological parameters of chlorophyll fluorescence for macroalgae, among other sea plants and photosynthetic marine invertebrates, are highlighted in Bhagooli et al. (2021a).

Mauritius is a tropical island situated in the South-Western Indian Ocean and has a high diversity of macroalgae, including 435 species reported: 108 green algae (Chlorophyta), 268 red algae (Rhodophyta), and 59 brown algae (Phaeophyta) (Jagtap 1993; Bolton et al. 2012; Mattio et al. 2013; Beetul et al. 2016). Macroalgal species which are most commonly available and which exhibit the highest antioxidant activities from each taxonomic class include *Turbinaria ornata* (Turner) J.Agardh, *Gracilaria salicornia* (C.Agardh) E.Y.Dawson (Somanah et al. 2012). Globally, reports are suggestive that these macroalgae can adjust at the biochemical level to cope with varying light levels under different thermal conditions, but such investigations remain limited and almost non-existent in Mauritius. It has previously been reported that *U. lactuca* L. has a net increase in photosynthesis and growth rate with augmenting temperature (Nejrup et al. 2013) and increasing light intensities (Geertz-Hansen and Sand-Jensen 1992; Zhang et al. 2020). Such responses have also been reported in other *Ulva* species. For example, when *Ulva pertusa* Kjellman was cultured in a PES/5 medium at 30°C for one week, it was found that cytoplasmic as well as vacuolar contents in the cell increased, the chloroplasts became dark green, and the cell wall thickening. In addition, the darkening of the chloroplasts at the higher temperature was consistent with the higher photosynthetic activity of the sterile mutant of *U. pertusa* at 30°C, which implies that *U. pertusa* undergoes physiological changes to withstand high temperature. The *G. salicornia* has broad tolerance potential to environmental changes such as temperature and irradiance, and it has also been shown that the photosynthetic responses differ with spatial distribution, for example, the macroalgae collected in Thailand had higher photosynthetic potential under conditions of strong light and high temperature than Japanese macroalgae (Phooprong et al. 2007). In a study on other red algae, it was found that among *Gracilaria* species, namely *G. arcuata, G. textorii, G. vermiculophylla, G. incurvata, G. foliifera, G. corticata, G. edulis, G. lichenoides*, only the rhodophye *G. vermiculophylla* exhibited a high-temperature tolerance limit of up to 35°C even though it normally had an optimum growth rate at 25°C. The T. ornata has exhibited high antioxidant activity and increased production of phenols with significant spatio-temporal variations. Other brown algae, such as *Fucus distichus*, can increase their photosynthetic performance and function at molecular and physiological levels due to thermal tolerance, including heat shock response and stress conditions. Several studies have been carried out on the macroalgal species from the Mauritian waters: ranging from their biodiversity (Bolton et al. 2012; Mattio et al. 2013), distribution (Jagtap 1993; Ramah et al. 2021a,b; Bhagooli et al. 2021b,c), photophysiology (Bhagooli et al. 2021d), impacts on corals (Kaullysing et al. 2016), antioxidant activities (Somanah et al. 2012; Gopeechund et al. 2020) based on seasonality (Ramah et al. 2014) to their use in aquaculture (Msuya et al. 2014) and public perception for a sustainable industry (Lutchmanen and Bhagooli 2015). Though increasing temperature and light level trends have been observed in the Mauritian coastal waters (Bhagooli and Taleb-Hossenkhan 2012; Mattan-Moorgawa et al. 2012; Bhagooli and Kaullysing 2019), reports on the differential responses and acclimation potential of local macroalgal species to these environmental changes are very limited. This study investigates the photosynthetic performance, phytochemical content and antioxidative responses of the three most commonly available macroalgal species from the Mauritian lagoons, *U. lactuca, G. salicornia* and *T. ornata*, to different temperature and light treatments in vitro and sets the basis for investigating the possible mechanisms behind any differential, adaptive responses as well as the conditions that may be optimal for future aquaculture practices (Gao and Beardall 2022).

MATERIALS AND METHODS

Sampling of macroalgal species

Approximately 2 kg of each marine macroalgae species, *U. lactuca*, *G. salicornia* and *T. ornata* (Figure 1) were

collected from different geographic regions of the Mauritius Island (Pointe aux Sables, Poste de Flacq, and Balaclava, respectively) (Figure 2) during summer in the month of December 2010 at a depth of 0.5 to 1 meter. The physico-chemical parameters of sampling sites are indicated in Table 1. The macroalgal species were identified according to the 'Guide to the Seaweeds of KwaZulu-Natal' (De Clerck et al. 2005) and Algaebase (Guiry and Guiry 2017). The specimens were carefully cleaned with filtered seawater to remove any debris, sand, mud, epiphytes, seagrasses or other macroalgae attached.

Set-up and experimental design

An in vitro experiment was set up to evaluate the photosynthetic performance, phytochemical contents (polyphenols) and antioxidant activities when exposed to different thermal and irradiance conditions (Table 2). Chlorophyll fluorescence measurements (Sadally et al. 2016; Bhagooli et al. 2021a,d) were taken and polyphenols were extracted (Bahorun et al. 2004) from the macroalgae to assess activity at different intervals: when freshly collected, after acclimatization and after treatment with different light and/or thermal regimes as (Figure 3).

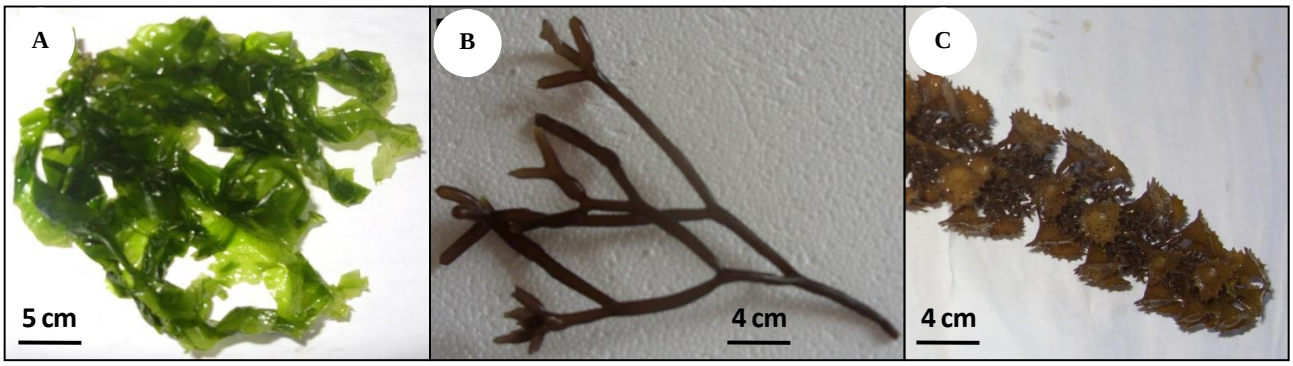

Figure 1. The three marine macroalgal test species A. *Ulva lactuca*, B. *Gracilaria salicornia*, C. *Turbinaria ornata*

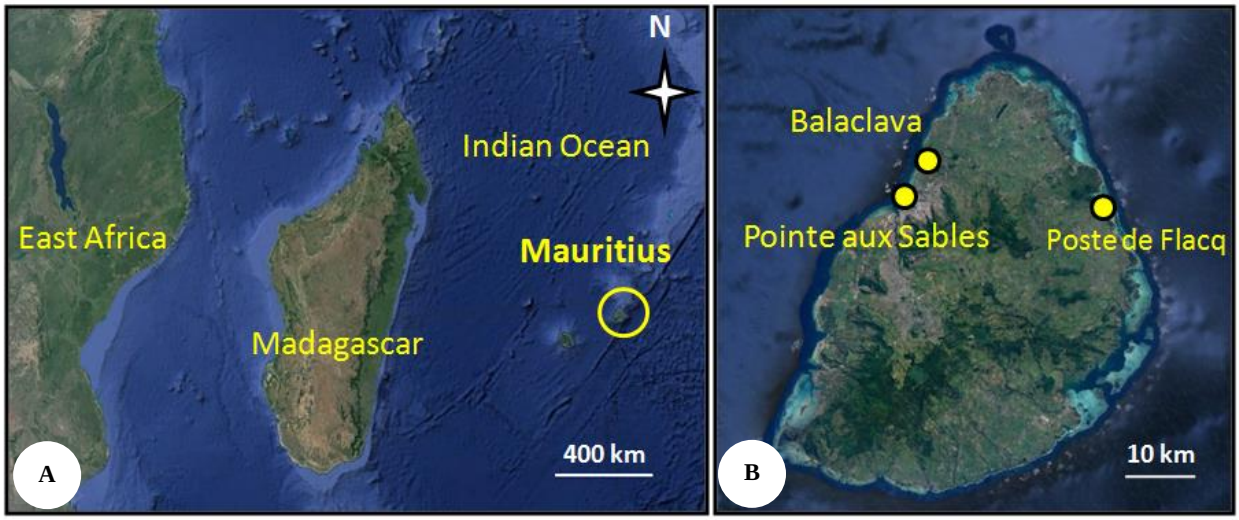

Figure 2. Satellite images of sampling sites. A. Mauritius Island is located in the South West Indian Ocean and B. The three macroalgal species, *U. lactuca*, *G. salicornia* and *T. ornata* were collected from different geographic regions of the Mauritius (Pointe aux Sables S20º10'13.2"E057º26'51.6", Poste de Flacq S20°09'39.70"E57°44'50.32", and Balaclava S20º04'47.1"E057º30'41.2", respectively) (Source: Google Earth 2015)

Table 1. Physico-chemical parameters at sampling sites for each collected macroalgal species. *U. lactuca*, *G. salicornia* and *T. ornata* were collected from Pointe aux Sables, Poste de Flacq and Balaclava, respectively

Parameters/species collected	*Ulva lactuca*	*Gracilaria salicornia*	*Tubinaria ornata*
Seawater temperature (°C)	29.0	26.0	28.0
Light intensity (µmol quanta $m^{-2}s^{-1}$)	2367.0	2330.0	2339.0
Dissolved oxygen(mg/L)	16.7	18.6	18.5
Conductivity (mS)	68.0	69.0	68.9
Salinity (ppt)	34.0	32.0	33.0
pH	8.12	8.41	8.32

Table 2. Temperature and irradiance conditions to which the macroalgal species were exposed

Control/ Stress conditions	Abbreviations	Light (12hr/ day) (µmol quanta $m^{-2}s^{-1}$)	Temperature (°C)
Control (same conditions as acclimatization): Control light, Control temperature	CLCT	1.55 ± 0.63	28
Condition 1: Control light, High temperature	CLHT	1.55 ± 0.63	32
Condition 2: Moderate light, Control temperature	MLCT	100 ± 63.6	28
Condition 3: Moderate light, High temperature	MLHT	100 ± 63.6	32

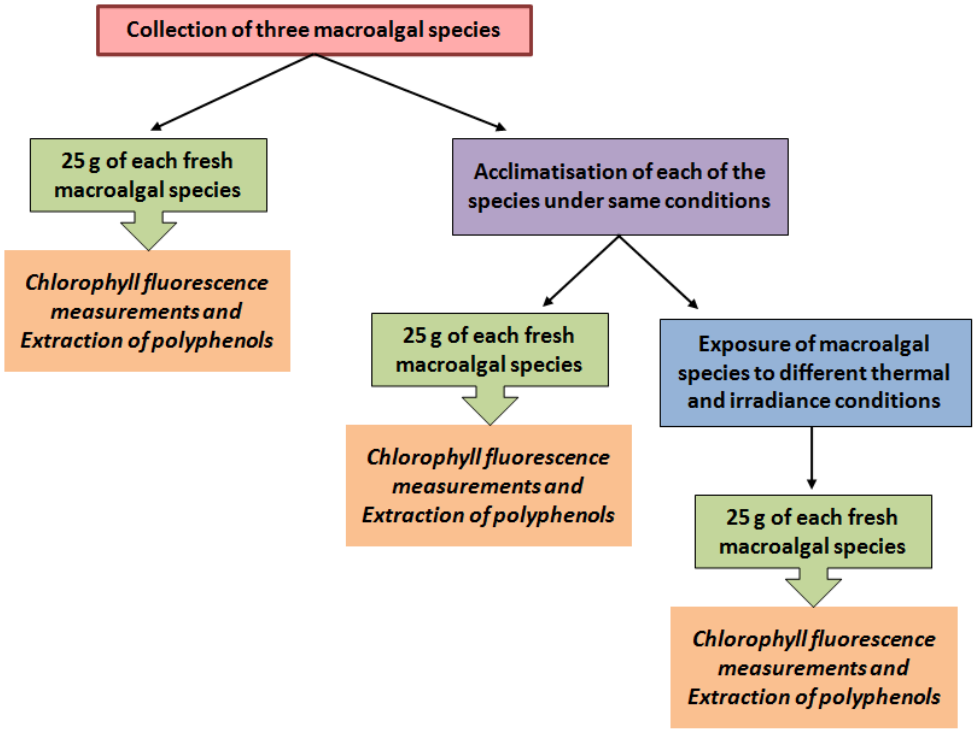

Figure 3. Flowchart representing the post-sampling treatments of macroalgal species and the intervals at which chlorophyll fluorescence measurements were taken and extraction of polyphenols was performed

A temperature of 28°C was used as control temperature since the *in-situ* temperature of seawater (site of collection) was 27.6°C and the stress temperature used was 32°C as it was the maximum temperature recorded in the Mauritian lagoons. Moderate light intensity (100 ± 63.6 µmol quanta $m^{-2}s^{-1}$) was provided by white light fluorescent tube bulbs that were just above the relevant aquaria. Low light intensity refers to ambient laboratory condition (1.55 ± 0.63 µmol quanta $m^{-2}s^{-1}$). In condition 1, only thermal stress was given; in condition 2, light stress was given to the macroalgae and in condition 3, both temperature and light stresses were given as a combined treatment. The physico-chemical parameters were measured at intervals of 7 days (Table 2).

The three macroalgal species were acclimatised, under same thermal and irradiance conditions, for 2 weeks (Tables 3) in aquaria containing filtered seawater. The systems were aerated using an artificial aerator (High quality fine tuning Air Pump AC-9906, ResunTM) and thermostatically controlled at 28°C (Automatic aquarium heater CB-8300, ResunTM) under ambient laboratory light conditions (1.55 ± 0.63 µmol quanta $m^{-2}s^{-1}$) and without duplication of any tidal cycle (Smolina et al. 2016). After acclimatization, thalli of the three macroalgal species were attached to nylon strings fixed on plastic tubings in an aquarium of 67L filtered seawater, aerated and thermostatically controlled. The same was done in triplicate (3 aquaria). Each triplicate was exposed to different thermal and irradiance conditions (Table 2 and Figure 4). The stresses were applied for a period of 7 days on a 12-hr basis per day (6 a.m. to 6 p.m.).

Determination of photosynthetic performance

The photosynthetic response was determined by chlorophyll fluorescence measurements (Smolina et al. 2016). Pulse-amplitude modulated (PAM) fluorometry (MINI-PAM: Walz, Germany) was used to assess the photo-physiology of the macroalgal species, by measuring the fluorescence of chlorophyll a thereby determining the relative electron transport rate (rETR) and non-photochemical quenching (NPQ) when exposed to a series of rapidly (10s) changing light climates (RLC) (McMinn et al. 2012). All the parameters were measured in triplicates using the PAM fluorometer which consisted of Emitter

Detector unit connected to a computer operated with WIN Control software (Heine Walz GmbH, Effeltrich, Germany). The distance between the specimens and the optic fiber head of the fluorometer was kept constant (10mm) for each sample so that accurate measurement could be achieved.

Following the RLCs' recordings, samples were dark-adapted for 30 minutes prior to the maximum quantum yield (Fv/Fm) measurements, which is an indicator of quantum efficiency. The variable fluorescence Fv obtained is the difference between the maximal fluorescence from the fully reduced PSII reaction center (Fm) and the initial fluorescence (Fo) from the antenna of fully oxidized PSII (Buchel and Wilhelm 1993; Hanelt 1998). The effective quantum yield (ΦPSII) was thus calculated according to Genty and colleagues (Genty et al. 1989).

$$YIELD = (Fm'-F)/Fm' = \Delta F/Fm'$$

The rETR and NPQ were estimated, at each increasing actinic light irradiance in 9 discrete increments, using the RLC and the values were plotted (Bhagooli et al. 2008; Louis et al. 2016). The double exponential decay function (Platt et al. 1980) was used to fit curves to the RLCs. The rETR was calculated as described by Schreiber and colleagues (1986).

$$rETR = 0.5 \times \Phi PSII \times PAR$$

Where:

PAR is the photosynthetically active radiationnce;

ΦPSII is the effective quantum yield and is calculated as: (Fm'— F)/Fm', where Fm' and F is the maximum and minimum fluorescence yield, respectively;

The '0.5' number in the equation accounts for 50% of absorbed photons used by PSII.

The NPQ is a process by which oxygenic photoautotrophs harmlessly dissipate excess light absorbed as heat and fluorescence (Szabò et al. 2005; Roth 2014;) and was derived from the expression (Fm-Fm')/Fm'. Fm is the maximal fluorescence of a dark-'adapted' sample and Fm' is the maximal fluorescence of a light-exposed alga under a given irradiance. NPQmax represents the highest NPQ value derived from the RLC.

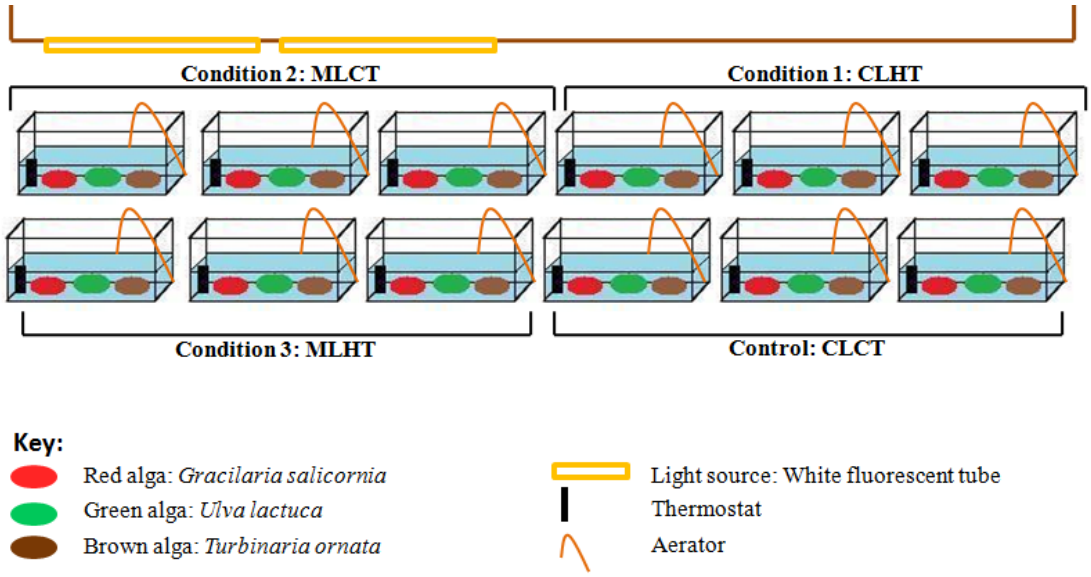

Figure 4. Diagrammatic representation of the experimental set-up used for application of temperature and light treatments on the three macroalgal species

Table 3. Parameters to which *Ulva lactuca*, *Gracilaria salicornia*, and *Turbinaria ornata* were acclimatized

Parameters	*Ulva lactuca*			*Gracilaria salicornia*			*Tubinaria ornata*		
	Period of acclimatization: 14 days								
	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14
Temperature (°C)	27.0	27.5	28.5	26.5	27.0	28.0	27.0	27.5	28.0
Light intensity (µmol quanta $m^{-2}s^{-1}$)	1.20	1.35	1.13	1.11	1.30	1.17	1.11	1.31	1.22
Dissolved oxygen (mg/L)	22.8	24.8	27.4	23.2	23.4	23.8	18.6	24.5	29.6
Conductivity (mS)	68.9	67.2	68.1	68.5	69.2	70.1	69.1	69.8	70.1
Salinity (ppt)	35.0	35.0	35.0	35.0	36.0	35.0	35.0	35.0	35.0
pH	7.73	7.84	7.88	7.78	7.62	7.59	7.89	7.51	7.21

Extraction of polyphenols and preparation of macroalgal extracts

The extraction of polyphenolics from the macroalgal species were carried out according to a modified protocol used by Bahorun et al. (2004). A total mass of 25g of the test macroalga was crushed in liquid nitrogen and the resulting fine paste was macerated in 80 mL of 80% methanol for 2 to 3 hours on an orbital shaker at room temperature. The mixture was filtered and the extraction step was repeated twice with the residue. The filtrates were pooled together, centrifuged (3000 rpm for 15 minutes, 25°C) and then filtered using vacuum pump. The resulting filtrate was concentrated in vacuo at 36°C in a rotary flash evaporator (Heidolph Laborata 4003). All concentrated macroalgal extracts were lyophilized and stored at -20°C until further analysis.

Determination of total phenol content

The total phenol content of methanolic extracts was determined using the Folin-Ciocalteu method which was adapted from Singleton and Rossi (1965). To a volume of 0.125 mL aqueous extract, 1.75 mL of distilled water and 0.25 mL of Folin-Ciocalteu reagent were added. After 3 minutes, 0.5 mL of 2% sodium carbonate was added followed by incubation at 40°C for 40 minutes. The blue coloration formed was read at 685 nm against water as blank standard using a spectrophotometer (Unicam Instruments, Cambridge, UK). The total phenol contents of the macroalgal species were expressed in mg of gallic acid equivalent/g dry weight.

Determination of total flavonoid content

The estimation of total flavonoid was done using the aluminium chloride method adapted from Lamaison and Carnet (1991). To 1 mL of methanolic extract, an equal volume of 1 mL of 2% (w/v) methanolic AlCl3.6H20 was added. The mixture was incubated at room temperature for 10 minutes and the resulting pale yellow coloration was read at 440 nm against methanol as a blank. The flavonoid contents were quantified with respect to quercetin standard curve and expressed in mg quercetin equivalent /g dry weight.

Determination of antioxidant activity

Ferric Reducing Antioxidant Potential (FRAP) assay

Estimation of the antioxidant capacity of the macroalgae species was performed using the Ferric Reducing Antioxidant Potential (FRAP) assay, adapted from Benzie and Strain (1996). This assay measures the ability of an antioxidant molecule in the seaweed extracts to reduce Fe^{2+}/tripyridyl-s-triazine complex to the blue colored ferrous form. Fresh FRAP reagent was prepared by mixing 10 mM TPTZ dissolved in 10 mL of 40 mM HCl, 10 mL of 20 mM Ferric chloride 100 mL of acetate buffer (0.25M; pH 3.6). To 100 µL of aqueous sample, 300 µL of distilled water was added followed by 3 mL of pre-warmed FRAP reagent (37°C). The samples were kept at room temperature for 4 minutes in the dark. The resulting blue colored solution was read at 593 nm, against water as blank standard. The results were expressed in terms of mMol ferrous sulphate equivalent /g dry weight.

Trolox Equivalent Antioxidant Capacity (TEAC) assay

Another method to estimate the antioxidant capacity of the macroalgal extracts is the Trolox Equivalent Antioxidant Capacity (TEAC) assay, adapted from Campos and Lissi (1996). This method assesses the ability of an antioxidant compound to scavenge the ABTS (2, 2'-Azino-bis (3-ethylbenzthiozoline-6)-sulfonic acid) radicals relative to that of the standard antioxidant Trolox. The ABTS+ radicals were generated by mixing 0.5mM ABTS and 1mM MnO_2 in 50 mL phosphate buffer (0.1M, pH 7). The reaction mixture was shaken for 15 minutes and the blue-green solution was filtered and kept on ice, in dark to prevent degradation. To 500 µL of aqueous macroalgal extract, 3 mL of $ABTS^+$ was added and the decay in absorbance at 734 nm was monitored for 15 minutes. Distilled water was used as blank. The difference between the final and initial absorbance values were used to calculate the antioxidant activity of the macroalgal extract. The results were expressed in terms of mMol Trolox equivalent /g dry weight.

Statistical analysis

All measurements were taken in three replicates. The data were analyzed using Microsoft Excel 2003 and are reported as mean ± standard error. Graphs were generated using GraphPad Prism (version 6.0). Spearman's correlations were determined using SPSS software (version 16.0). Three-Way Analysis of variance (ANOVA) was performed after arcsin (square root) transformation of all raw data using STATISTICA software (version 10.0) to evaluate the effects of temperature and irradiance on the photosynthetic performance (yield, rETRmax and NPQmax), phytochemical contents (phenols and flavonoids) and antioxidant activities (FRAP and TEAC) of the 3 macroalgal species. The Post-hoc Tukey Honestly Significant Difference (HSD) analysis was then used to assess differences between means at 5% level of significance (p-values <0.05 were considered to be statistically significant).

RESULTS AND DISCUSSION

Photosynthetic responses of the three macroalgae to thermal and irradiance conditions

Photosynthetic yield (Fv/Fm) ranged from 0.18 ± 0.00 to 0.94 ± 0.03, relative electron transport rate (rETRmax) from 1.65 ± 0.11 to 89.30 ± 2.70 and non-photochemical quenching (NPQmax) from 0.06 ± 0.09 to 1.83 ± 1.02. Temperature significantly affected all the three photosynthetic parameters measured (Table 4). The *T. ornata* appeared to be most affected whereby a significant decrease ($p<0.001$) in photosynthetic yield (Fv/Fm) was observed when the species was exposed to thermal stress, that is under CLHT and MLHT treatments (Figure 5). Interestingly, *U. lactuca* and *G. salicornia* showed slight and non-significant decrease in photosynthetic yield when

exposed to thermal and light stresses. The *U. lactuca* exhibited significant increase in relative electron transport rate under all treatments ($p<0.001$), notably underthe MLCT condition (89.3 ± 4.67). The *G. salicornia* responded in the opposite way, whereby the relative electron transport rate declined significantly in all treatments ($p<0.001$), with most pronounced decrease in the MLHT condition (1.65 ± 0.186). The *T. ornata* underwent a significant decrease in electron transport rate when exposed to high thermal stress (CLHT and MLHT conditions) and relative to control condition (CLCT), a significant increase ($p<0.001$) was observed with increase in light stress (MLCT) (34.8 ± 15.6). Remarkably, non-photochemical quenching activity in *U. lactuca* and *G. salicornia* was higher when exposed to stress conditions (MLCT and CLHT, respectively) as compared to when these species were freshly collected. Relative to control, the non-photochemical quenching activity in *U. lactuca* with a significant and pronounced increase ($p<0.001$) under the MLCT condition. The *G. salicornia* showed similar response under the CLHT condition. In *T. ornata*, the non-photochemical quenching dropped significantly ($p<0.001$) upon exposure to thermal and light stresses, with the most decrease under the MLCT condition.

Phytochemical responses of the three macroalgae to thermal and irradiance conditions

Total phenol contents ranged from 0.47 ± 0.06 to 9.58 ± 0.64mg GAE/ g DW (Figure 6A) and total flavonoid contents ranged from 3.62 ± 0.31 to 76.80 ± 5.64 mg quercetin equivalent/ g DW (Figure 6B), which varied significantly between species ($p<0.001$) (Table 4). Across freshly collected species, the orders of highest to lowest levels of phenols and flavonoids are *T. ornata*>*G. salicornia*>*U. lactuca* and *T. ornata*>*U. lactuca*> *G. salicornia*, respectively. Total phenol contents of all species decreased when acclimatized. Relative to the control condition (CLCT), the total phenol contents in *U. lactuca* increased significantly under stress conditions, that is under CLHT ($p<0.01$), MLCT ($p<0.05$) and MLHT ($p<0.05$). Interestingly, the phenol contents of the macroalga under stress were higher than when freshly collected. The total phenol contents of *G. salicornia* and *T. ornata* dropped when exposed to different thermal and light stresses and both species appeared to be most affected under the MLHT condition. While the differences in phenol content were significant in *G. salicornia* ($p<0.01$ under CLHT, $p<0.05$ under MLCT and $p<0.01$ under MLHT), those in *T. ornata* were not significant.

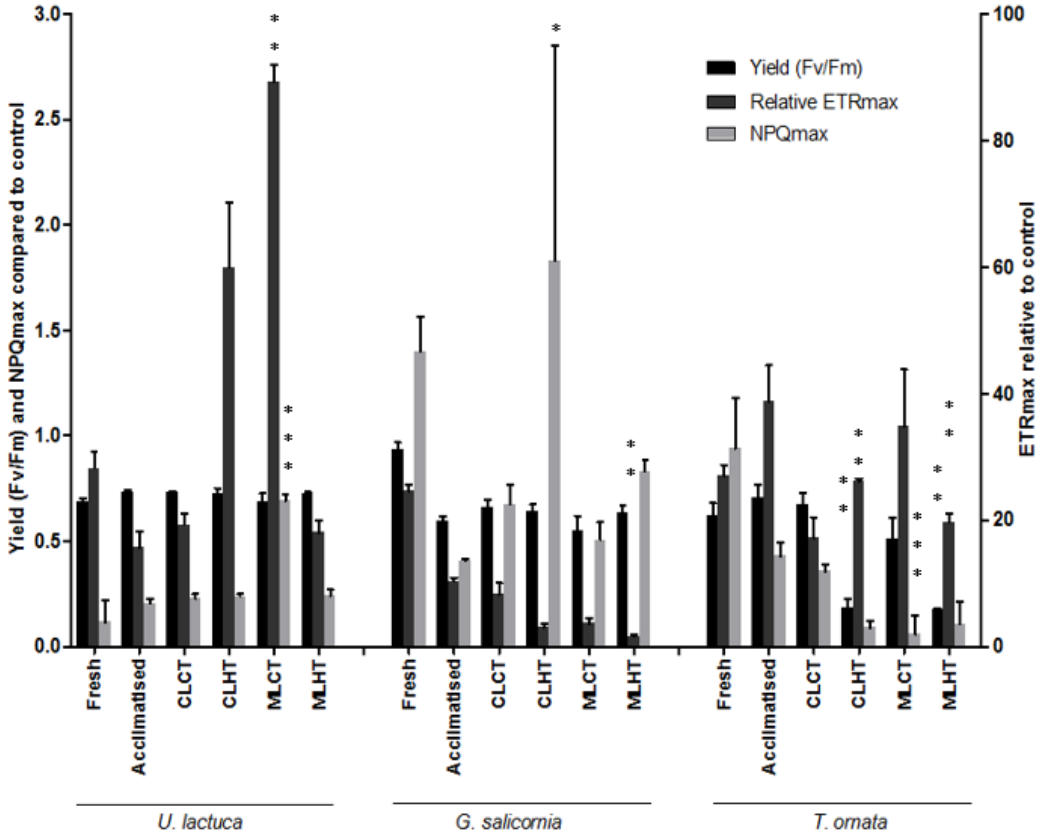

Figure 5. Photosynthetic performance in terms of chlorophyll fluorescence measurements: Fv/Fm(yield), rETRmax and NPQmax of the three macroalgal species when freshly collected, acclimatised for 2 weeks and when exposed to temperature and light treatments for 1 week, compared to control (Mean ± standard error, n=3). Significant differences between control values (CLCT) and values under the stress conditions (CLHT, MLCT, MLHT) of each species are indicated by asterisks over the stress condition bar. (*** = $p<0.001$; ** = $p<0.01$; * = $p<0.05$)

Table 4. Three-Way ANOVA for effects of temperature and light on photosynthetic performance (yield, electron transport rate and non-photochemical quenching), phytochemical contents (phenol and flavonoid contents) and antioxidant activities (FRAP and TEAC values) of the three macroalgal species. Asterisks indicate significant differences (p-values) at 5% level (n=3). (*** = p<0.001; ** = p<0.01; * = p<0.05)

Parameters	Dependent variables	Source of variation	df	MS	F-value	p-value
Photosynthetic performance	Maximum Yield (F_v/F_m)	Species	2	0.4437	107.42	0.000***
		Temperature	1	0.0536	12.99	0.000***
		Light	1	0.0010	0.24	0.628
		Species x Temperature	2	0.0227	5.50	0.005**
		Species x Light	2	0.0016	0.39	0.676
		Temperature x Light	1	0.0000	0.00	0.966
		Species x Temperature x Light	2	0.0003	0.08	0.928
	Relative electron transport rate ($rETR_{max}$)	Species	2	1.55622	224.160	0.000***
		Temperature	1	0.08222	11.844	0.000***
		Light	1	0.01386	1.997	0.161
		Species x Temperature	2	0.03363	4.844	0.010*
		Species x Light	2	0.01694	2.440	0.093
		Temperature x Light	1	0.25391	36.574	0.000***
		Species x Temperature x Light	2	0.10081	14.521	0.000***
	Non-photochemical quenching (NPQ)	Species	2	1.70144	84.091	0.000***
		Temperature	1	0.08234	4.070	0.047*
		Light	1	0.01923	0.950	0.332
		Species x Temperature	2	0.13828	6.834	0.001**
		Species x Light	2	0.02103	1.039	0.357
		Temperature x Light	1	0.00868	0.429	0.514
		Species x Temperature x Light	2	0.06956	3.438	0.036*
Phytochemical contents	Total phenol contents	Species	2	45912.6	23.62337	0.000 ***
		Temperature	1	1419.7	0.73048	0.401
		Light	1	2778.3	1.42954	0.244
		Species x Temperature	2	2738.3	1.40892	0.264
		Species x Light	2	1632.9	0.84015	0.444
		Temperature x Light	1	483.2	0.24860	0.623
		Species x Temperature x Light	2	162.0	0.08334	0.920
	Total flavonoid contents	Species	2	6296366	78.3088	0.000***
		Temperature	1	23576	0.2932	0.593
		Light	1	252033	3.1346	0.089
		Species x Temperature	2	1708	0.0212	0.979
		Species x Light	2	357449	4.4456	0.022*
		Temperature x Light	1	245977	3.0593	0.093
		Species x Temperature x Light	2	70309	0.8744	0.430
Antioxidant activities	FRAP	Species	2	124388.3	153.4509	0.000***
		Temperature	1	0.3	0.0004	0.984
		Light	1	2693.9	3.3234	0.081
		Species x Temperature	2	0.4	0.0005	0.999
		Species x Light	2	2720.8	3.3565	0.052
		Temperature x Light	1	1643.6	2.0277	0.167
		Species x Temperature x Light	2	1622.5	2.0016	0.157
	TEAC	Species	2	319738289	490.4683	0.000***
		Temperature	1	45540970	69.8584	0.000***
		Light	1	52890880	81.1329	0.000***
		Species x Temperature	2	45982264	70.5353	0.000***
		Species x Light	2	54708169	83.9206	0.000***
		Temperature x Light	1	3310623	5.0784	0.033*
		Species x Temperature x Light	2	2890347	4.4337	0.023*

The total flavonoid content in *U. lactuca* decreased significantly when exposed to the three thermal and light stresses (p<0.001 under CLHT, p<0.001 under MLCT, and p<0.05 under MLHT) and appeared to be most affected under high-temperature stress, that is, the CLHT condition. Despite this decrease, the flavonoid amounts in *U. lactuca* under the stress conditions were higher than when freshly collected. Another interesting observation was that flavonoid contents in *U. lactuca* were attained under the control condition (CLCT). The total flavonoid contents of *G. salicornia* varied when exposed to thermal and light stresses. In contrast, there was a significant increase in

flavonoid contents under the CLHT condition (p<00.1), and a slight and significant decrease was observed under MLCT (p<0.001) and MLHT (p<0.01) conditions. The *T. ornata* showed a slight increase in total flavonoid contents under high thermal stress (CLHT and MLHT conditions) and a sharp decrease with increasing light stress (MLCT condition), with no significant difference from the control condition.

Antioxidant activity of the three macroalgae when exposed to thermal and irradiance conditions

Antioxidant activity varied significantly across species (p<0.001). FRAP values ranged from 0.002 ± 0.00 to 2.22 ± 0.04 mM ferrous sulphate /g DW (Figure 7A) and TEAC values ranged from 0.004 ± 0.00 to 0.21 ± 0.02 mM Trolox equivalent /g DW (Figure 7B). FRAP and TEAC assays indicated that *T. ornata* exhibited the highest antioxidant activities, followed by *U. lactuca* and *G. salicornia*. Differential responses were observed when the macroalgal species were exposed to light and thermal stresses. Both assays showed a significant increase in antioxidant activity of *U. lactuca* under all stress conditions (p<0.001), and the highest increase occurred under the CLHT condition. FRAP assay showed a decrease in antioxidant activity of *G. salicornia* and *T. ornata* when exposed to the stress conditions and they appeared to be most affected under the MLCT and CLHT condition, respectively. TEAC assay showed a similar decrease, however, *T. ornata* appeared to be most affected under the MLHT condition. When *T. ornata* was exposed to the highest stress condition (MLHT), the antioxidant activity declined and it was observed that the thalli of *T. ornata* turned dark and degraded into a powdery form. *G. salicornia* exhibited lower antioxidant activity than *U. lactuca, yet they could* withstand the thermal and light conditions morphologically. While light showed no significant change in antioxidant capacity, the temperature had a significant effect on species (p<0.001) (Table 4).

Correlation between phytochemical performance, phytochemical composition, and antioxidant activities of the three macroalgae

To our knowledge, the relationship between photosynthetic performance, phytochemical contents, and antioxidant activities in the three macroalgae is being investigated for the first time. Yield and NPQ values indicated a negative relationship with phytochemical contents (Phenol, r=-0.21; Flavonoid, r=-0.63 and Phenol, r= -0.31; Flavonoid, r= -0.41, respectively) and antioxidant activities (FRAP, r=-0.49; TEAC, r=-0.36 and FRAP, r=-0.31; TEAC, r=-0.26, respectively). ETRmax values showed a positive correlation to phytochemical contents (Phenol, r=0.42; Flavonoid, r=0.14) and antioxidant activities (FRAP, r=-0.03; TEAC, r=0.07). Both FRAP and TEAC values showed stronger correlations to flavonoid contents (r=0.909, r= 0.845, respectively) than to phenolic contents (r=0.688, r=0.758, respectively).

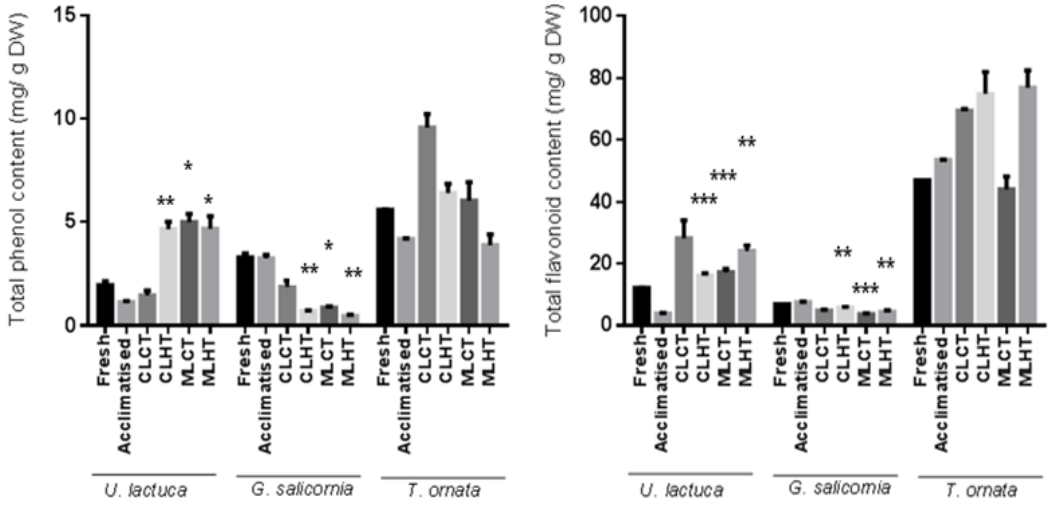

Figure 6. Phytochemical contents. A. Total phenol contents (mg Gallic acid equivalent/g dry weight), B. Total and flavonoid contents (mg Quercetin equivalent/ g dry weight) of the three species of macroalgae when freshly collected, when acclimatized for 2 weeks, and when exposed to temperature and light treatments for 1 week, compared to control (Mean ± standard error, n=3). Significant differences between control values (CLCT) and values under each species' stress conditions (CLHT, MLCT, MLHT) are indicated by asterisks over the stress condition bar. (*** = p<0.001; ** = p<0.01; * = p<0.05)

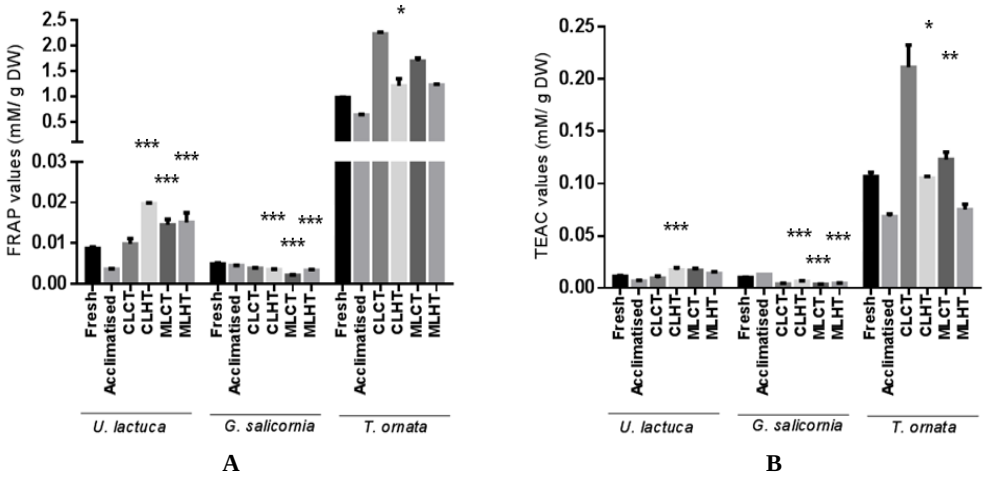

Figure 7. Antioxidant activity. A. Ferric reducing antioxidant potential (FRAP) (mM Ferrous sulfate equivalent/ g dry weight) and B. Trolox Equivalent Antioxidant capacity (TEAC) (mM Trolox equivalent/ g dry weight) values of the three species of macroalgae when freshly collected, when acclimatized for 2 weeks and when exposed to temperature and light treatments 1 week, compared to control (Mean ± standard error, n=3). Significant differences between control values (CLCT) and values under each species' stress conditions (CLHT, MLCT, MLHT) are indicated by asterisks over the stress condition bar. (*** = $p<0.001$; ** = $p<0.01$; * = $p<0.05$)

Discussion

This study showed that in vitro temperature and irradiance impacted the photosynthetic performance, phytochemical contents, and antioxidant activities of the tested macroalgae. This study contributes to the plethora of literature aiming at understanding the complexity of responses exhibited by species of macroalgae to environmental conditions. However, it remains difficult to understand the exact mechanisms since each species reacts differently to different stresses. Furthermore, changes in conditions typically considered as "stressors" may, under some levels and in certain species, instead enhance performance and survival. For instance, while increasing temperature and irradiance is more stressful for certain species, other species can physiologically withstand such changes.

Effect of thermal and irradiance on photosynthetic response of the three macroalgae

Differences in photosynthetic performances were observed in the tested macroalgae regarding photosynthetic yield, relative electron transport rate, and non-photochemical quenching (NPQ). Macroalgae can acclimate to changing irradiances through several mechanisms. One such mechanism is photo-inhibition, a high light-induced reduction of the photosynthetic quantum yield and increased NPQ of excess excitation (Lambrev et al. 2012; Zhang et al. 2020). This fact was supported by our findings, whereby under high irradiance stress, U. lactuca, followed by *G. salicornia*, exhibited higher NPQ than *T. ornata*. Depending on the species, this protective process can occur through the synthesis/activation of D1 protein or the xanthophyll cycle (Häder et al. 2002). The D1 protein largely regulates the reaction center of photosystem II, the main photo-inhibition site, by controlling the electron transport after primary photon absorption. Under excessive light intensity, the rate of synthesis of the D1 protein diminishes, leading to a decline in the number of active PSII centers. This decreases the maximum efficiency of PSII primary photochemistry (Fv/Fm) (Chow et al. 1989). Inactivation or destruction of the D1 protein of PSII might have led to the collapsing of active PSII centers of *T. ornata*, hence turning black and *G. salicornia*, which therefore turned yellowish green and bleached. This observation is consistent with reports indicating that climatic changes can impact negatively on macroalgal physiology (Ji and Gao, 2021) affecting, influencing the photosynthetic rate, growth (Li et al. 2013), and level of bleaching (Xiao et al. 2015). Another protective pathway to cope with excess light is the xanthophyll cycle through heat dissipation of excess excitation energy preventing the formation of singlet oxygen in the chloroplasts (Nan et al. 2008; Jahns and Holzwarth 2012). The Xanthophyll cycle involves the carotenoid pigments like violaxanthin, antheraxanthin and zeaxanthin. During light stress, conversion of violaxanthin to zeaxanthin through antheraxanthin provides photoprotection by acting as a lipid-protective antioxidant and stimulating non-photochemical quenching (NPQ) within the light-harvesting proteins, hence aggregation of Light-Harvesting Complex II, protecting the PSII reaction centers against photo-damage. This can explain the increased NPQ in *U. lactuca* and *G. Salicornia* as well as the reduced Fv/Fm in *T. ornata* at MLHT condition. It is reported that the xanthophyll cycle is the main NPQ mechanism in *Ulva* spp. during the first acclimation period to high light intensity (Eismann et al. 2020).

Temperature is among the most important environmental factors which generally mediate macroalgae distribution, growth, and productivity (Li et al. 2020) because varying temperatures can alter macroalgae enzyme activity, regulating physiological metabolism and

ultimately affecting photosynthesis and growth (Shi et al. 2021). For instance, a study showed that when the brown alga *Fucus vesiculosus* was incubated at warmer seawater temperatures, elevated maximum quantum yield of PSII, a decrease in NPQ, and an increase in the relative growth rate compared to algae incubated under ambient conditions were observed (Colvard and Helmuth 2016). In the current study, similar observations were made in the tested brown macroalgae, *T. ornata*, whereby there was a drastic decrease in NPQ with increasing temperature. Such observations may indicate the vulnerability of brown algae to high temperatures. On the other hand, an increase in temperature led to an increase in NPQ in *U. lactuca*, followed by *G. salicornia*, which explains their ability to acclimate to such stress. Beyond the optimum temperature, metabolic rates of macroalgae decline due to limitations in enzyme capacity (Atkin and Tjoelker 2003).

Increased temperature can also be associated with a limitation on the activity of Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco RuBisCO), an important enzyme involved in the light-dependent reactions of photosynthesis, via inactivation of the enzyme or by limited RuBisCORubisco activity and photorespiration. There is a decrease in RuBisCO activity at high temperatures, whereas there is an increase in pigment levels (Davidson 1991). When exposed to high temperature or high irradiance conditions, an increase in NPQ in *U. lactuca* and *G. salicornia* may indicate that there has been an increase in pigments and an eventual increase in photosynthetic performance, as a form of adaptation to changing environmental conditions. It has also been reported that macroalgae increase their composition and cellular abundance of light-harvesting pigment-protein complexes and hence ratios of PSI:PSII reaction centers and of other catalysts within the electron transport chain and changes in the abundance of Calvin-Benson cycle enzymes, most notably RuBisCO when exposed to high irradiance (Machalek et al. 1996).

The decrease in the RuBisCO enzyme also indicates that there is no need for additional nitrogen, which it uses for protein synthesis, indicating that nutrient is not a limiting factor. This evidence supports our observations whereby *U. lactuca*, documented for high nutrient uptake rates (Scanlan et al. 2007; Gao et al. 2014), showed no physiological damage related to nutrient uptake. Heat shock response is also one of the mechanisms of thermal tolerance. Most heat shock proteins (HSPs) are molecular chaperones that help organisms to ameliorate stress-induced changes by refolding denatured cellular proteins and degrading/replacing proteins that cannot be repaired (Hofmann and Todgham 2010). Thus, HSPs are the universal biomarkers of environmental stress (Smolina et al. 2016). Heat shock response largely depends on the expression of HSP hsp genes. Although hsp genes are expressed as a general response to a variety of physiological stresses (Feder and Hofmann 1999), the best-studied response is thermal activation, which occurs in response to temperature increases of 5°C to10°C greater than the average environmental temperature experienced by an organism (Lindquist 1986). In the current study, the temperature increase from the natural environment to the high-temperature treatment was nearly 5°C, which might have triggered the production of HSPs in the macroalgal species.

Our findings indicate that *U. lactuca* is the most tolerant species, followed by *G. salicornia* and *T. ornata* to in vitro environmental stresses. Acclimation to changing environmental conditions can be both beneficial and detrimental. The benefits of tolerance to high environmental stress include sustaining the biodiversity of the species and promoting their use in aquaculture. Preliminary studies in Mauritius indicated *G. salicornia* and *U. lactuca* as potential candidates for animal diet and *U. lactuca* as having high potential as plant growth promoters. Several food products, including pickles and jams, have been developed from *G. salicornia* and *U. lactuca* by the Agricultural Research and Extension Unit (AREU) in Mauritius, and preliminary data have shown positive feedback from consumer acceptability surveys (Mauritius Research and Innovation Council, 2013) and hence signifies a great potential for the future of macroalgae farming in the Western Indian Ocean (Msuya et al. 2014). In the same line, *U. lactuca* is the most robust and documented for high market value (food, medical products, feed supplement, fertilizers, and biofuel) (Yu-Qing et al. 2016) and can be a potential species to be considered for further cultivation.

Our findings, complemented with further investigations, can help to enhance the cultivation conditions in terms of the most favorable temperature and irradiance conditions. The problem associated with thermal and irradiance tolerance of species may include algal blooms, which may be detrimental to other marine organisms, probably leading to a shift in their distribution. This statement is supported by many studies that consider green algae, such as *U. lactuca*, to bloom owing to their physiological features such as rapid uptake of nutrients, enhanced growth rate, and wide environmental tolerance (Smayda 1997; Taylor et al. 2001).

Effect of thermal and irradiance conditions on phytochemical contents of the three macroalgae

Marine macroalgae produce a diverse array of chemicals involved in cellular defense, increasing their chance of survival in highly competitive environments (Kelman et al. 2012). Macroalgae, growing in stressful conditions under intense exposure to UV radiation, have developed protective mechanisms and have been recognized as an important source of secondary metabolites and macromolecules with antioxidant activity (Tziveleka et al. 2021). More than 40 years of research into the macroalgal natural products' chemistry and chemical defenses have led to more than 15,000 novel compounds, many of which have been shown to have bioactive properties (Cardozo et al. 2007; Barbosa et al. 2014). The present study assessed the abundance of phytochemical contents concerning thermal and irradiance conditions. Across species, the highest phenol and flavonoid contents were observed in *T. ornata*, followed by *G. salicornia* and *U. lactuca* when freshly collected. Upon

exposure to increasing stress (MLHT), phenol content increased only in *U. lactuca*, and flavonoid content varied across species. Another study indicated that generally, as water temperature increases, the phenolic contents of macroalgae decrease (Vergeer et al. 1995). In this study, *U. lactuca* appeared to be an exception since it behaved differently. It has also been documented that climatic changes can negatively impact macroalgal physiology by affecting the production of chemical defenses through changes in phenolic content (Vergeer et al. 1995).

In this study, more flavonoids were yielded as compared to phenols. Phenols are very sensitive to light and can get oxidized very easily. Thus, it can be said that with time, phenolic compounds degrade, leading to an underestimation of the phenolic contents of the algal species. This study performed total flavonoid quantification prior to phenolic content estimation. This can be a reason for the low yield of phenol contents. It has been documented that phenolic content also increases as thalli age (Stiger et al. 2004), thereby decreasing palatability with reproductive maturity and providing chemical defense for mature floating thalli (Cronin and Hay 1996). Since the thalli of *T. ornata* used in this study were very mature, this may be why they exhibited the highest phenol contents but could not sustain high stress beyond a certain level. Therefore, the highest flavonoid content was observed in *T. ornata*. Since flavonoids compose of several hydroxyl groups on the outside of the benzene ring, they are expected to exhibit a radical scavenging effect or sometimes to have a prooxidant effect as a source of ROS (Tagliaferro et al. 2002). Flavonoids are synthesized via the shikimate pathway, and their biosynthesis is affected by nutrient availability, altitude, water, genetic variation in composition, depending on species, and most importantly, temperature and light.

Effect of light and thermal stress on antioxidant activities of the three macroalgae

The effect of ROS in photosynthetic organisms is aggravated by excessive light. This is because excess energy input can increase the concentration of excited electrons, which can eventually oxidize many other molecules. Therefore, organisms have developed a wide range of protective mechanisms that eliminate reactive species, produced as by-products of photosynthesis and photo-oxidative events, before they cause any harm to sensitive parts of the cellular machinery (Foyer and Shigeoka 2011). These can be classified as low molecular weight compounds (phytochemicals such as carotenoids, melatonin, reduced glutathione, mycosporine like-amino acids) and enzymatic catalysts of high molecular weight (enzymes like catalase, superoxide dismutase). Phytochemicals like carotenoids not only maximize the spectrum of photosynthetically active radiationPAR but also protect the light-harvesting pigments in the antenna against phytochemical damage caused by excited triplet states (Carvalho et al. 2004).

Chloroplasts have membranes rich in polyunsaturated fatty acids (PUFAs), which are potential targets for peroxidation (Gutteridge and Halliwell 1990). Enhancing chloroplast antioxidant defenses has proved to be one of the most effective ways of protecting cells from abiotic stress (Ishikawa and Shigeoka 2008). Reactions involving antioxidants in macroalgae are complex and depend on the physico-chemical properties of the test reagents and substrates. Thus, it is recommended that the evaluation of antioxidant activity should employ more than one method to provide complementary assessments that consider different mechanisms that confer the antioxidant activities (Sahidi 2006). On this basis, two independent assays were employed: FRAP and TEAC. Antioxidant activity varied significantly across species ($p<0.001$). While light showed no significant change in antioxidant capacity, the temperature significantly affected the antioxidant activity of the macroalgal species ($p<0.001$).

Marine macroalgae consist of internal biological clocks that control the time when different physiological processes should occur (Carvalho et al. 2004). Therefore, many ROS-generating processes are slow under normal conditions, while under environmental factors, like high light, algae are stressed, and the generation of these radicals can be accelerated. A higher level of antioxidants is thus vital to withstand photo-oxidative stress excited by a reducing energy-consuming capacity (Carvalho et al. 2004). In a study by Aguilera and colleagues (Aguilera et al. 2002), higher antioxidant enzymatic activities and higher amounts of antioxidant compounds were measured in green algae compared to red and brown algae, indicating a more efficient biochemical protection in algae exposed to higher stress conditions. Overall, the ecological success of macroalgae, be it in the eulittoral or upper sublittoral, is partly due to an enhanced reactive oxygen scavenging mechanism, promoting fast acclimation to the changes in environmental radiation conditions. As temperature and light increase, a decrease in humidity is brought about, which favors photosynthesis and eventually quenching more radicals. These results are comparable to the present study, whereby *U. lactuca* showed higher photosynthetic efficiency, polyphenolic contents, and antioxidant power when exposed to the high-stress condition. *U. lactuca* showed greater tolerance to stress conditions than the other tested macroalgal species. This may be why chlorophytes are the most preferred for aquaculture (Moreira et al. 2021). Compared to other algal taxa, antioxidant enzyme activities in brown algae are low (Aguilera et al. 2002).

Evaluation of enzymatic antioxidant enzymes was not performed in this current study but it is important to investigate the reason why even though *T. ornata* exhibited high polyphenolic content and eventual high antioxidant power attributed to the phytochemicals, yet could not withstand the high-stress condition and collapse morphologically. Therefore, investigation of primary and secondary metabolites (pigments), enzymatic antioxidant system (catalase, superoxide dismutase, GPX) as well as protein expression (HSPs, D1 protein) on these macroalgal species will help understand further the defense mechanisms exhibited under environmental stress. Other factors crucial in understanding stress tolerance include considering non-photosynthetic aspects of metabolism (for

example, respiration), growth rate, stress duration, life stage/age of the organism, nutrient uptake, synthesis of important proteins (for example, D1 and HSP), pigment content (chlorophyll), enzymatic and non-enzymatic system.

In conclusion**,** the present study indicated that in vitro thermal and irradiance stresses affect the photosynthetic performance, phytochemical contents, and antioxidant activities of *U. lactuca*, *G. salicornia*, and *T. ornata*. The differences in responses of the macroalgae to the light and temperature treatments may have been due to their morphology (surface area and lifecycle of organism), protein/enzymatic inactivation or damage, growth rate (efficiency of nutrient uptake), and defense mechanisms (enzymatic and non-enzymatic antioxidant systems, heat shock response). Though an organism exhibits high antioxidant powers, it does not necessarily imply that the same organism may be able to withstand a high-stress condition in vitro and, most probably, in its natural habitat as well. Our results are suggestive that an increase in seawater temperature around Mauritius could be detrimental to some macroalgal species and eventually affect surrounding marine organisms. The growth of macroalgae is fundamentally regulated by temperature and light, and knowing a species' physiological response to these parameters is vital to predicting future macroalgae distributions. A further in-depth investigation is warranted in order to understand the protective biochemical mechanisms that help macroalgae against thermal and irradiance stresses, notably the enzymatic antioxidant system and the gene expression biomarkers, as well as to determine the light and temperature conditions that different macroalgae can sustain for application in aquaculture practices and adaptation to a globally changing marine environment.

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Diplosoma simile (Ascidiacea: Didemnidae) distribution and its photosynthetic thermal stress responses from Mauritius: Implications for invasive or opportunistic behavior

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Abstract. *Fai ALY, Kaullysing D, Jeetun S, Soondur M, Bhagooli R. 2023.* Diplosoma simile *(Ascidiacea: Didemnidae) distribution and its photosynthetic thermal stress responses from Mauritius: Implications for invasive or opportunistic behavior. Indo Pac J Ocean Life 7: 16-26.* This study aimed to investigate the morphological identity of the ascidian *Diplosoma*, its distribution, experimental thermal stress responses, and its characteristics of being potentially invasive or opportunistic in Mauritian waters. The *Diplosoma* species were anesthetized in menthol, fixed, and preserved in a formalin-seawater solution. Isolated zooids and embryos were examined under a microscope for distinct morphological characteristics, which revealed their identity as *D. simile* (Sluiter, 1909). The *D. simile* percentage cover studied at seven sites in 2019 around Mauritius tended to be high at the near-reef zone, with a high percentage of dead coral cover per 0.5 m^2. At Flic en Flac, long-term observations in 2008, 2010, 2016, and 2019 indicated a significant decrease and increased from 61.25±3.31 to 6.15±0.60 % and 0.08±0.05 to 2.50±090% for the percentage covers of live coral and *D. simile*, respectively. The *D. simile* was recorded on five coral species, and its percentage occurrence increased from 2008 to 2019 in *Acropora muricata, A. cytherea*, *Pocillopora damicornis*, and *Montipora aequituberculata* with the highest levels occurring in *Acropora* while *Porites lutea* was not found to be covered by *D. simile* during the study. Visual observations from the thermal exposures at 27ºC, 30ºC, and 33ºC during 19 hrs revealed that *D. simile* was more affected by increasing temperature and exposure time. Using four observed conditions of normal, bleached/paled, tissue sloughing, and mortality, *D. simile* suffered from only bleaching/paling at 33ºC at 19 hrs exposure. Variable visual responses were noted for corals, with *P. lutea* appearing normal at all trials while *A. cytherea* was suffering from mortality both at 30ºC and 33ºC at 19 hrs exposure. The effective quantum yield (ΦPSII), measured using a Diving-PAM, declined significantly at 33ºC treatments at 6 and 19 hrs of exposure. *D. simile* generally tended to be more thermally tolerant than corals like *P. lutea*, *P. damicornis*, *M. aequituberculata, A. muricata, A, cytherea*, though the ΦPSII thermal responses were variable among the tested corals. When considering the reported behavioral characteristics of *D. simile*, namely the lack of broad dispersal range and wide environmental tolerance, it is tempting to deduce that the species is potentially invasive. However, in this study, *D. simile*'s significant distribution on dead corals, its presence in zones of high anthropogenic activities, and its relatively more robust thermal stress responses than corals colonized suggest an opportunistic behavior.

Keywords: Ascidian, corals, *Diplosoma simile*, distribution, diving-PAM, Mauritius, opportunistic behavior, thermal tolerance

INTRODUCTION

Ascidians emerge from the kingdom Animalia and belong to the class Ascidiacea, which is considered the largest class of the Subphylum Tunicata (Phylum Chordata) (Hirose and Hirose 2013). These organisms have been subject to many studies regarding their bioactive compounds and broad physico-chemical tolerance, which provides for their complex behavioral patterns (Hirose and Hirose 2013; Hirose and Nozawa 2010). Furthermore, ascidians have further been reported to display characteristics that significantly increase risks of invasion outbreak, that is, their globally widespread distributions and broad environmental tolerances (Hirose and Hirose 2013; Akram et al. 2015; Koplovitz et al. 2015; Villalobos et al. 2017).

However, knowing that these organisms have extensive dispersal ranges and are easily overlooked, it would not be surprising that many occurring species are left uncharted (Goodbody 2000; Hirose et al. 2012; Villalobos et al. 2017). Around Mauritius Island, for instance, only 32 species have been reported around the Island (Table 1) with 2 species being endemic, namely: *Pseudodistoma mauritiana* and *Polycarpa nigricans*. However, the discovery of another ascidian species with high morphological similarity to the genus *Diplosoma* around Mauritius Island has led to considering the high possibility of a documentation gap regarding ascidians. With increasing evidence of coral vulnerability worldwide, it is crucial to understand and address any further potential threats posed by ascidians (Carilli et al. 2010; Pandolfi et al. 2011; Bhagooli and Taleb-Hossenkhan 2012).

Coral bleaching and reef degradation rates are escalating worldwide and locally, mostly due to increasing global temperatures and exposure to anthropogenic-related stressors (Mattan-Moorgawa et al. 2012; Louis et al. 2016; 2020; Hughes et al. 2017; Bhagooli and Kaullysing 2019; Bhagooli

et al. 2021a) and some reefs are predicted to be "locally extinct" (Sheppard 2003; Bhagooli and Sheppard 2012). It is generally considered that corals bleach, whereby they lose their zooxanthellae and/or the zooxanthellae photosynthetic pigments (Glynn 1993), and this disassociation between the coral animal and its dinoflagellate zooxanthellae occurs in most cases due to the zooxanthellae photosynthetic dysfunctioning under thermal and light stressors (Iglesias-Prieto et al. 1992; Warner et al. 1996; 1999; Jones and Hoegh-Guldberg 1998; Bhagooli 2013). Knowing that such phenomena are indicators of decreasing coral reef health status, the latter is more at risk of being subject to our competition by reef-associated organisms, which would further reduce chances of coral recovery (Mattan-Moorgawa et al. 2018; Bhagooli and Kaullysing 2019). Moreover, the urgency of in-depth studies on ascidian-related threats is even more pressing given that ascidian-induced-bioinvasion has been increasingly reported worldwide, especially following mass thermal-induced coral bleaching (Dijkstra et al. 2007; Tebbett et al. 2019). It is noteworthy that several local studies on photo-physiological responses to thermal and other stressors have been conducted on scleractinian corals (Mattan-Moorgawa et al. 2015, 2018; Kaullysing et al. 2016; Louis et al. 2020; Bhagooli et al. 2021b), seaweeds (Narain et al. 2023; Bhagooli et al. 2021c) and seagrasses (Bhagooli et al. 2021c), microalgae (Sadally et al. 2016; Soondur et al. 2021, 2022) ascidians thermal stress responses are yet to be investigated.

Several authors further mentioned the high invasiveness of the ascidian genus *Diplosoma* (Sommer et al. 2010; Rodriguez-Martinez et al. 2012; Hirose et al. 2012), some reporting it to smother and kill live *Acropora* spp., which are the preferred host species, following their settlement on areas of dead corals (Littler and Littler 1995; Li et al. 2016). To avoid any confusion arising from the controversial and often misleading nature of the similar terms "invasive" and "opportunistic" species, the present study referred to Colautti and MacIsaac (2004) definition of invasive species and Whitlatch and Zajac's (1985) definition of opportunism.

The present study aimed at investigating the extent of the ascidian species' invasive potential around Mauritius Island. The objectives included: (i) Morphological characterisation of the ascidian species; (ii) Assessment of the distribution of the ascidian around Mauritius relative to the status of the coral health; (iii) Evaluation of the thermally-induced photosynthetic stress response of the ascidian species relative to some of its coral hosts; and (iv) Inference from these investigations on the potential invasive or opportunistic behavior of the ascidian in the Mauritian context.

MATERIALS AND METHODS

Field surveys

Field surveys were conducted at Flic Flac to determine the long-term percentage cover of live coral and *Diplosoma* at Flic en Flac in October/November 2008, 2010, 2016, and 2019. Four stations along the reef flat at Flic en Flac were surveyed using five 1 m x 1 m quadrats per station. *Diplosoma* on five coral species, namely *Acropora muricata*, *A. cytherea*, *Pocillopora damicornis*, *Montipora aequituberculata*, and *Porites lutea* by observing 20, 10, 15, 5, and 5 coral colonies during each year's survey and expressed as percentage occurrence out of the observed colonies.

The percentage ascidian cover (%AC), dead coral cover (%DCC), and healthy coral cover (%HCC) were determined through field surveys in the summer between October 2019 and March 2020 at several sites around Mauritius. Following Leujak and Ormond (2007), the survey was performed at 7 selected sites around Mauritius Island: Balaclava, Trou aux Biches, Flic en Flac, Bel Ombre, Blue Bay Marine Park, Belle Mare, and Flat Island (Figure 1). Three zones were also selected at each site (based on their respective distance to the shore), namely: near-coast (20 m), lagoon (60 m), ad near-reef (150 m) zones. At each zone, a 20 m transect line was laid parallel to the shoreline, and a 50 cm x 50 cm PVC-made quadrat was photographed perpendicularly to the substratum at 0.5 m intervals along the line.

Microscopic observations

The methods described are based on Lafargue and Wahl (1987) and Marks (1996) protocols for examination under an optical microscope. The ascidian species samples were collected at Flic en Flac while still attached to their substrate. They were maintained in seawater-filled Ziploc® bags until reaching the University of Mauritius. First, the colonies were placed in a glass container in an aquarium with running water and left for 1 day to promote zooid relaxation. Next, 3.5 g/L menthol crystals were added and left unstirred to cause the least mechanical stress until the colonies were fully anesthetized (approximately 2 hrs). Next, the container was left in a freezer for 3hrs until the ice began to form before transferring to a solution of 10% formalin-seawater for 24 hrs. Finally, the colonies were transferred to a 7% formalin-seawater solution and kept for 4 weeks before microscopic observation.

Masson's acid Hemalum staining agent was prepared following Lafargue and Wahl (1987). First, 5 g of Aluminium potassium sulfate KAl(SO4)2 was dissolved in 100 mL of distilled water. Next, 0.2 g of Hematoxylin was added to the boiling solution and left to boil for a few more seconds before being cooled to room temperature. The solution was filtered and 2% acetic acid was added until a color change from dark purple to red was noticed. The staining agent was kept in the absence of light and would be valid a month post-preparation.

Fixed colony samples were thoroughly rinsed in distilled water before adding Masson's acid hemalum. First, the translucent colonial ascidian was immersed in the staining agent until the desired level of stain was achieved (~5 minutes). Next, the sample was rinsed with distilled water to remove all excess staining agents. Next, individual zooids and embryos were isolated from the colony and examined for morphological characteristics under an inverted microscope. The presence of calcareous spicule was further tested by immersing the colonial ascidian in 4% Hydrochloric acid (HCl), which would decalcify the calcareous content hence leaving small bubbles to form on the surface of the colony.

Table 1. List of ascidian species and their respective taxonomic classifications reported around Mauritius and other distribution locations in the Indo-West-Pacific Region

Suborder, family	Species name	Location	References
Aplousobranchia			
Synoicidae	*Pseudodistoma mauritiana**	Flic en Flac (Mauritius)	Vasseur (1967)
Didemnidae	*Didemnum candidum*	Australia, Malaysia, Indian Ocean, Trou aux Biches (Mauritius)	Monniot and Monniot (2001); Vasseur (1967)
	D. molle	Ile D'Ambre (Mauritius)	Mattan-Moorgawa et al. (2015)
	Atriolum robustum	Mayotte, Mauritius, Indian ocean	Monniot and Monniot (2001); Vasseur (1967)
	Tridemnum sansibaricum	Zanzibar, Mozambique, Flic en flac (Mauritius)	Monniot and Monniot (2001); Vasseur (1967)
	T. natalense	Australia, South Africa, Trou d'eau Douce (Mauritius)	Monniot and Monniot (2001); Vasseur (1967)
	T. neridionale	Trou aux Biches (Mauritius)	Monniot and Monniot (2001); Vasseur (1967)
	Diplosoma multifidum	Mauritius, India, Indonesia	Millar (1975)
Polycitoridae	*Eudistoma rhodopyge*	South Africa, South-west of Madagascar, Ilot Barkly (Mauritius)	Monniot and Monniot (2006); Vasseur (1967)
	E. mobiusi	Mozambique, Tanganyika, Zanzibar, South of Madagascar, Pointe d'Esny (Mauritius)	Monniot and Monniot (2006); Vasseur (1967)
	E. atrum	Mauritius	Monniot and Monniot (2006); Vasseur (1967)
	E. reginum	Mauritius	Monniot and Monniot (2006); Vasseur (1967)
	Polycitorella pallida	Mauritius	Monniot and Monniot (2006); Vasseur (1967)
	Cystodytes solitus	Mauritius	Monniot and Monniot (2006); Vasseur (1967)
	C. dellechiajei	Mauritius, Philippines, Australia	Millar (1975)
Clavelinidae	*Clavelina enormis*	Mauritius	Millar (1975)
Polyclinidae	*Aplidium convergens*	Mauritius	Monniot and Monniot (2006); Vasseur (1967)
	Polyclinum constellatum	Mauritius	Monniot and Monniot (2006); Vasseur (1967)
	P. festum	Mauritius	Millar (1975)
Phlebobranchia			
Ascidiidae	*Ascidia sydneiensis*	South Africa, Australia, Flic en Flac (Mauritius)	Palomino-Alvarez et al. (2019); Vasseur (1967)
	A. munda	North Australia, Flic en Flac (Mauritius)	Palomino-Alvarez et al, (2019); Vasseur (1967)
Stolidobranchia			
Styelidae	*Symplegma viride*	East and South Africa, Madagascar, Australia, Port Louis (Mauritius)	Vasseur (1967); Monniot and Monniot (2007)
	Amphicarpa inhacae	Mozambique, Ilot Barkly (Mauritius)	Vasseur (1967)
	Polyandrocarpa anguinea	South Africa, Port Louis (Mauritius)	Vasseur (1967); Monniot and Monniot (1994)
	P. tincta	Mozambique, Port Louis (Mauritius)	Vasseur (1967)
	*Polycarpa nigricans**	Cassis (Mauritius)	Vasseur (1967); Monniot (2002)
	P. madagascariensis	Mauritius, Mozambique, Madagascar	Monniot (2002)
	Cnemidocarpa madagascariensis	South-West Madagascar, Flic en Flac (Mauritius)	Vasseur (1967); Monniot (2002)
	Styela partita	Port Louis (Mauritius)	Vasseur (1967)
Pyuridae	*Pyura pulla*	Trou d'eau Douce (Mauritius)	Vasseur (1967)
	P. momus	Port Louis (Mauritius)	Vasseur (1967)
	Microcosmus exasperatus	Port Louis (Mauritius)	Vasseur (1967)
	Cynthia pallida	Mauritius, Jamaica, western pacific	Monniot (2002)
Molgulidae	*Molgula natalensis*	Ilot Barkly (Mauritius)	Vasseur (1967)

Note: **P. mauritiana* and *P. nigricans* are endemic ascidian species to Mauritius Island

Thermal stress experiment

Forty-five fragments, nine per each of the 5 colonies, of the ascidian *Diplosoma* on dead coral part and five coral species, namely *P. lutea*, *M. aequituberculata*, *Acropora muricata*, and *A. cytherea* were collected at Flic en Flac on 17th January 2020. Nine aquaria were set up and arranged in 3x3 order and labeled as 27°C (control), 30°C and 33°C in dim light conditions. Oxygen pumps were provided for each aquarium supplied with 5 fragments per species. All the tanks were further equipped with thermometers and representative tanks with Hobo temperature and light data loggers to monitor temperature and light levels.

Chlorophyll-a fluorescence measurement

The effective quantum yield at photosystem II (ΦPSII) was determined using a Diving-PAM (Pulse amplitude Modulator). A saturating pulse of 4000 µmol quanta m^{-2} s^{-1} and a weak light emission of <1 µmol quanta m^{-2} s^{-1} were used to measure the maximum (Fm) and minimum (Ft) fluorescence, respectively. ΦPSII was then calculated as (Fm-Ft)/ Fm (ΔF/ Fm) (Genty et al. 1989).

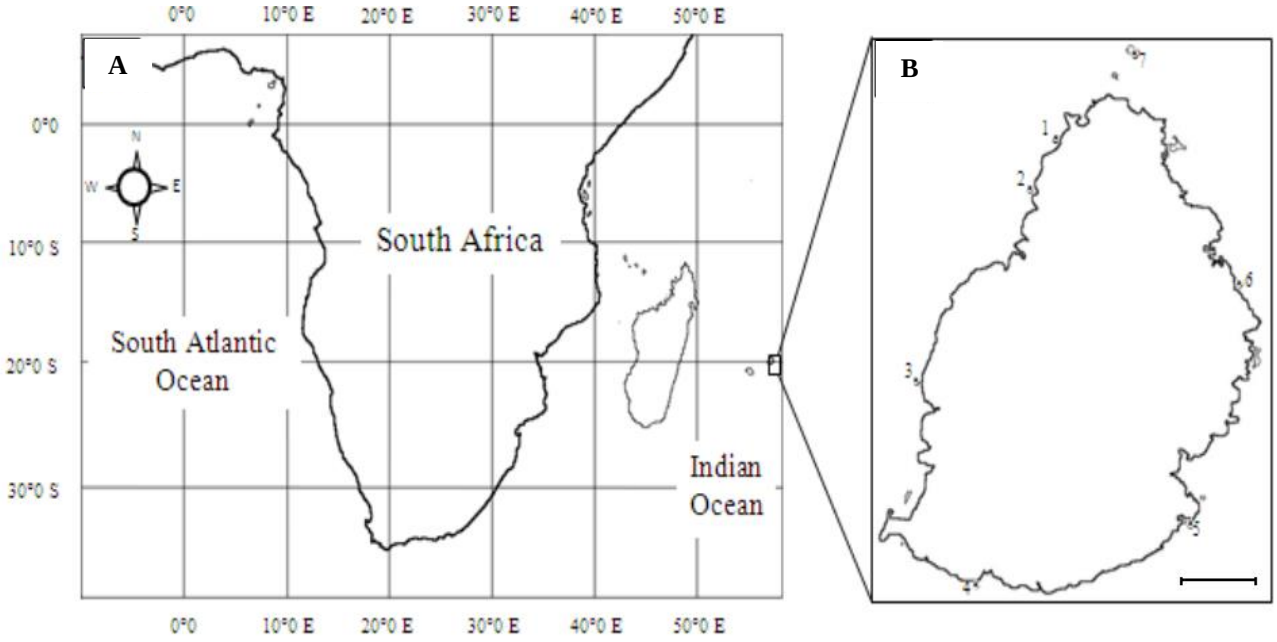


Figure 1. A. Map of the Indian Ocean showing the location of Mauritius (Scale = 10 km); B. Map of the study area. The numbers represent the snorkeling sites around Mauritius Island. 1. Balaclava, 2. Trou aux Biches, 3. Flic en Flac, 4. Bel Ombre, 5. Blue Bay, 6. Bellemare, 7. Flat Island

Ascidians and coral samples were left to shortly acclimatize for 3 hrs in seawater at room temperature (~27°C) prior to exposures at 27°C, 30°C and 33°C for a duration of 19 hrs. It is noteworthy that previous coral thermal stress studies have employed temperatures of 33ºC (Ralph et al. 2001) and 34, 36, and 38ºC (Warner et al. 1996) over periods of hours to days. ΦPSII was measured at times T_0, T_3, T_6, and T_{19} for initial 3 hrs, 6 hrs, and 19 hrs exposures, respectively, for all temperature trials. ΦPSII is one of the four main chlorophyll fluorescence parameters used in many studies investigating the photophysiology of sea plants and photosynthetic symbiotic marine invertebrates (Bhagooli et al. 2021d).

Data analysis

Using the software package PASW Statistics, the Shapiro-Wilk Normality test was used to assess the data distribution pattern. Correlations between variables were investigated through Spearman Rho's non-parametric test since none of the variables were normal. Principal Correspondence Analysis (PCA) was further performed on data regarding the species' distribution to investigate any correlation between percentage ascidian cover (%AC) and their location around the Island. Kruskal-Wallis and comparative Wilcoxon rank-sum test were computed to compare the percentage of ascidian cover relative to the percentage of dead coral cover (%DCC), percentage of healthy coral cover (%HCC), and distance from the shore. Similar statistical tests were run to compare the species ΦPSII relative to increasing temperature and exposure time.

RESULTS AND DISCUSSION

Ascidian morphological features: External features

The ascidian colonies were mostly found on *Acropora* sp., distributed on the underside of the coral structures already covered by seaweeds (indicating that these were dead before settlement). Although well hidden, they were spotted by their bright green color, which is due to the presence of *Prochloron*, a cyanobacterial symbiont having a dominant concentration of chlorophyll-a (Hirose et al. 2004; Hirose et al. 2012; Stalin et al. 2016). The 1mm-thick ascidian colonies varied in terms of colony size per coral structure. When exposed to a bright light source, they seemed to reflect as bright blue spots (Figure 2).

Ascidian morphological features: Internal features

No bubbles of gas were spotted on the surface of the colony sample upon exposure to (HCl) which implied that no spicules were present in the colony. Zooids were about 700 µm long (Figure 3) and were evenly distributed throughout the colony on the upper part of the tunic. Four rows of stigmata were also noticed, and the stigmal arrangement of each of the four rows from top to bottom formed the pattern "6-6-6-5". The species also lacked an atrial aperture in its thorax while having a two-lobed testis in its lower abdomen region. Furthermore, the ascidian's retractor muscle separated almost directly from under its thorax. Embryos of various sizes were also isolated (with the largest ones being 500-600 µm long), and only their sensory vesicle was noticed. Contrary to zooids, present mostly in the upper-tunic region, the embryos were unevenly present on the underside of the tunic (i.e., closest to the coral substrate).

Field surveys of live corals and ascidians at Flic en Flac

Field surveys conducted at Flic Flac for the years 2008, 2010, 2016, and 2019 revealed a significant decrease and increase from 61.25±3.31 to 6.15±0.60 % and 0.08±0.05 to 2.50±090% for the percentage covers of live coral and the ascidian species, respectively (Figure 4A). The survey on the occurrence of the latter ascidian on five coral species indicated that the percentage occurrence of the ascidian increased from 2008 to 2019 in *A. muricata*, *A. cytherea*, *P. damicornis*, and *M. aequituberculata* with the highest levels found in both the *Acropora* while *P. lutea* was not found to be covered by the ascidian during the study (Figure 4B). The *D. simile* occurred in four out of the five studied corals (Figure 5).

***D. simile* distribution pattern**

Principal Correspondence Analysis (PCA) (Figure 6) showed the highest % ascidian cover at near-reef zones as well as at sites known to harbor high anthropogenic activity, namely: Flat Island, Flic en Flac, and Balaclava (in decreasing order of correlation strength).

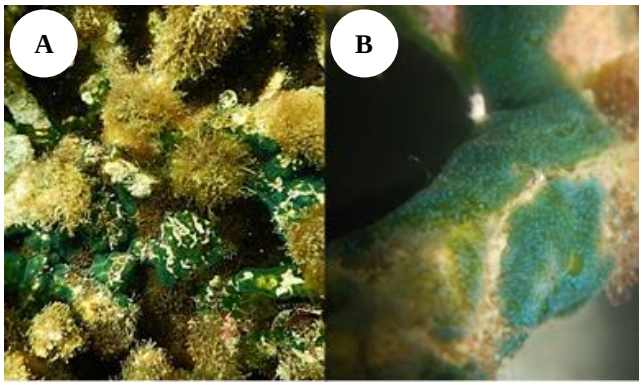


Figure 2. Ascidian species are growing on *Acropora* at Flic en Flac; A. Sample species in situ before collection; B. Sample species in a laboratory, showing natural bright green color after exposure to a camera flash

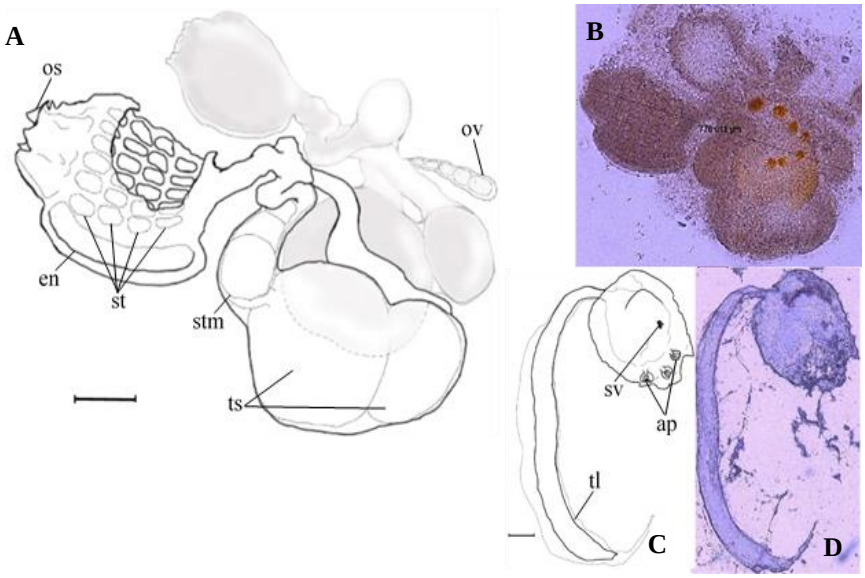


Figure 3. Isolated ascidian species at various life stages (Scale = 100 µm). A. Line drawing of an adult zooid; with one of the two individuals being shaded to facilitate morphological differentiation; B. Adult zooid (778 µm long); C. Line drawing of embryo; D. Embryo (459 µm long); (st) Stigmata; (os) Oral siphon; (en) Endostyle; (stm) Stomach; (ov), Ovary; (tl) Tail; (ad) Adhesive papillae; (sv) Sensory vesicle

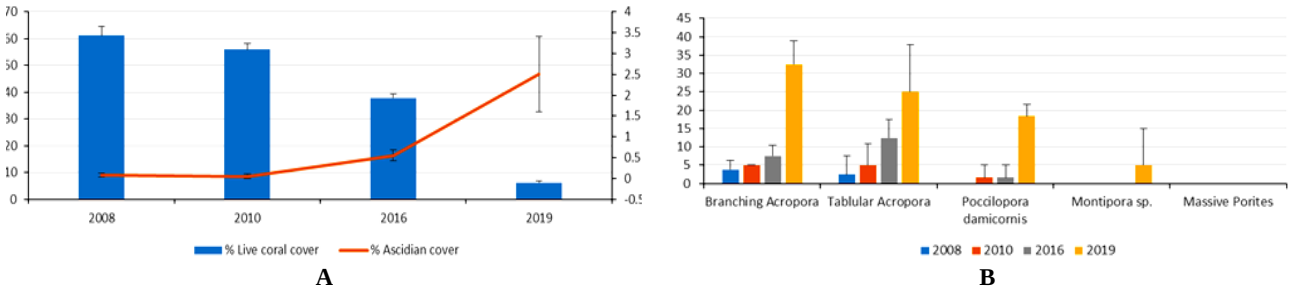


Figure 4. Percentage live coral and *D. simile* cover (A) and percentage occurrence of *D. simile* on coral species including branching *Acropora* (*A. muricata*), tabular *Acropora* (*A. cytherea*), cauliflower coral (*Pocillopora damicornis*), foliose coral (*Montipora aequituberculata*), and massive coral (*Porites lutea*) at Flic-en-Flac in years 2008, 2010, 2016 and 2019 (B)

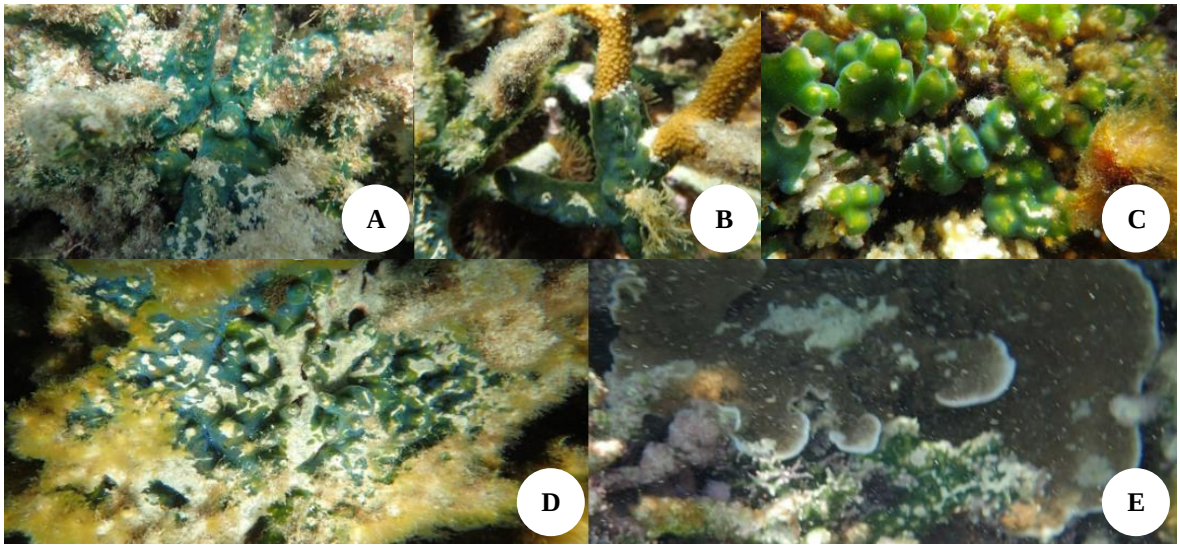


Figure 5. *D. simile* occupying completely dead corals and/or dead parts of live corals. A. Completely dead *Acropora muricata*; B. Live *A. muricata*; C. *Pocillopora damicornis*; D. Completely dead *A. cytherea*; and E. *Montipora aequituberculata*

The highest mean of percentage ascidian cover (%AC) was recorded at Flat Island (10.34 ± 1.16) and the lowest one at Blue Bay Marine Park (0.01 ± 0.01). The correlation between %AC and distance from shore yielded an R-value (+0.478) and a Kruskal-Wallis (P< 0.001), which indicated the presence of a significant correlation between both variables. PCA further revealed an interesting relationship between %AC, %DCC and %HCC, whereby %AC is positively related to %DCC and negatively related to %HCC. These relationships were confirmed with yielded R-values (+0.697) and (-0.359) for both tests, respectively as well as Kruskal-Wallis (P<0.05) in both cases. Such a finding suggests a highly significant correlation between %AC/%DCC and %AC/%HCC.

Effective quantum yield responses to thermal stress

The three thermal trials had temperature means and light intensity maximums of 26.8±0.5ºC and 1000.0lux; 30.4±0.3ºC and 882.6lux; and 33.7±0.6C and 958lux, respectively (Figure 7). The effective quantum yield at Photosystem II (ΦPSII) of *D. simile* significantly declined at 3 hr, though slightly, and at 19 hr in 33ºC trials (Figure 8A). The ΦPSII responses to thermal stress were variable among corals tested (Figure 8B-F), with *Acropora* (Figure 8E, F) being the most susceptible and *P. lutea* (Figure 8B) the least susceptible to thermal stress. Decreases in ΦPSII in *D. simile* were lower at 30ºC compared to *A. muricata*, *M. aequituberculata*, and *P. lutea* after 19hrs (Figure 9A). Generally, *D. simile* appeared to be more resistant than the test corals to thermal stress exposure in this study.

Thermal stress responses: Visual observations

The colonies' external color remained constant during the first 6hrs of the experiment at all temperatures; Only those maintained at 33ºC showed a slight decrease in the level of blue reflection. However, at time T=19 hrs, ascidians were surprisingly discovered to turn translucent grey from a bright green color. The colonies' surface areas having changed color were also estimated to be proportional to the temperature they were exposed to. Ascidians at 27ºC had a minimal <5% while those at 33ºC had about 50% change in color. When looking at four observed conditions of normal, bleached/paled, tissue sloughing, and mortality, *D. simile* suffered from only bleaching/paling at 33ºC at 19 hrs exposure (Figure 9B). The corals had variable visual responses, with *P. lutea* appearing normal at all trials while *A. cytherea* was suffering from mortality at 30ºC and 33ºC at 19 hrs exposure (Figure 9B).

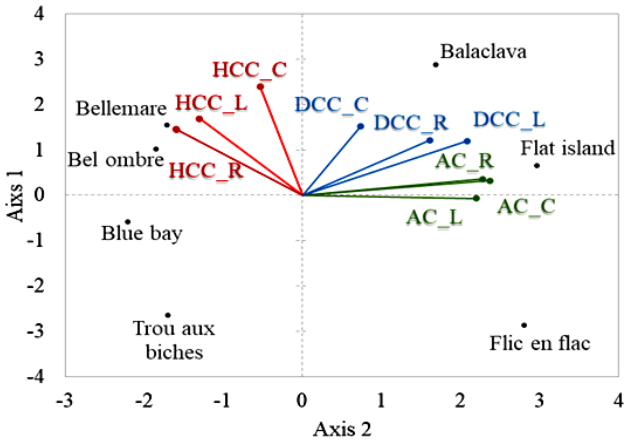
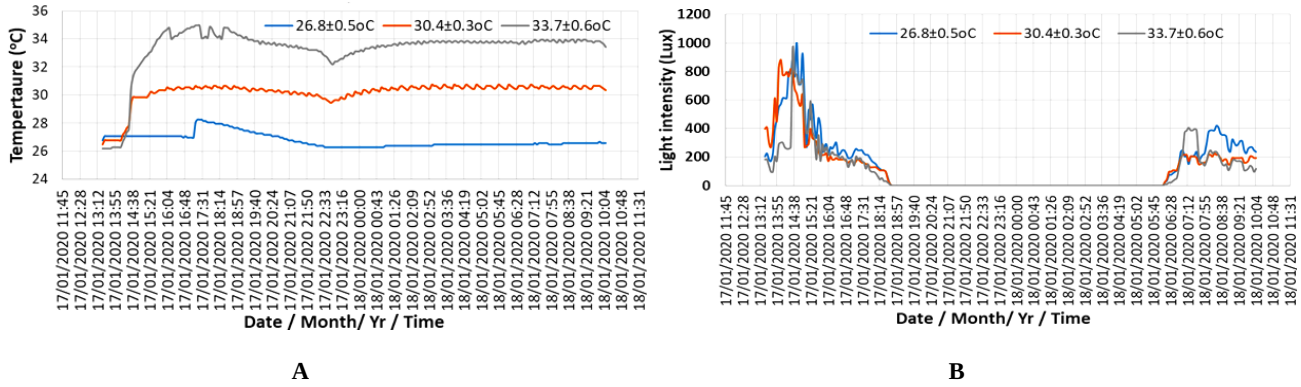
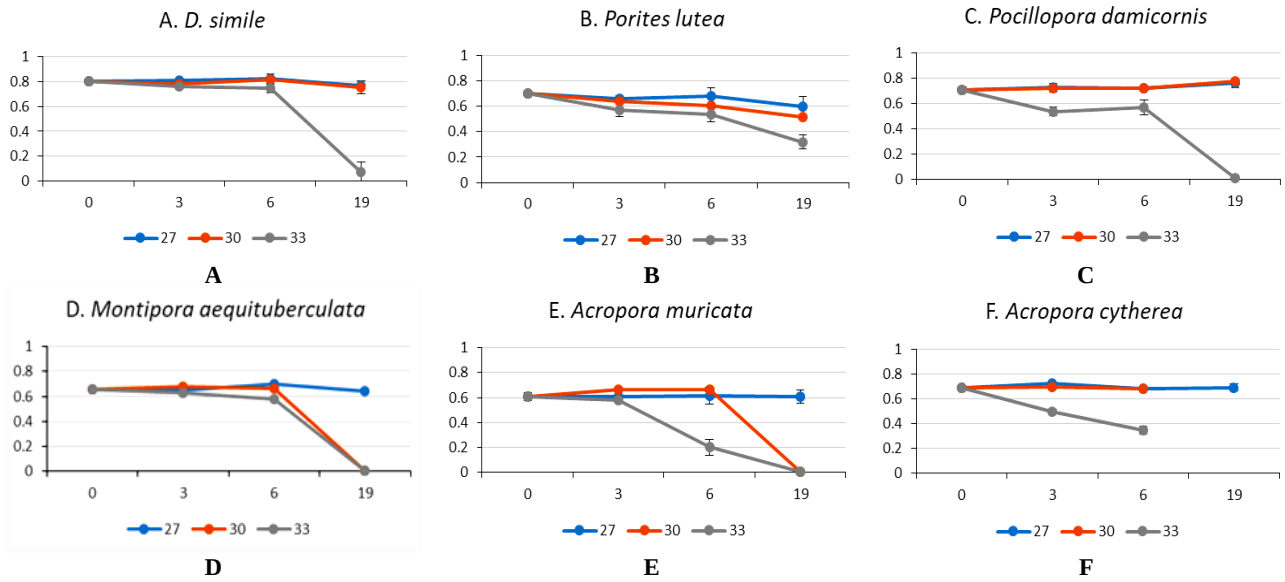


Figure 6. PCA indicates the relationships between variables like%AC, %DCC, %HCC, distance from shore, and location around the Island. (AC_C: %AC at coast, AC_L: %AC at lagoon, AC_R: %AC at reef)



A **B**

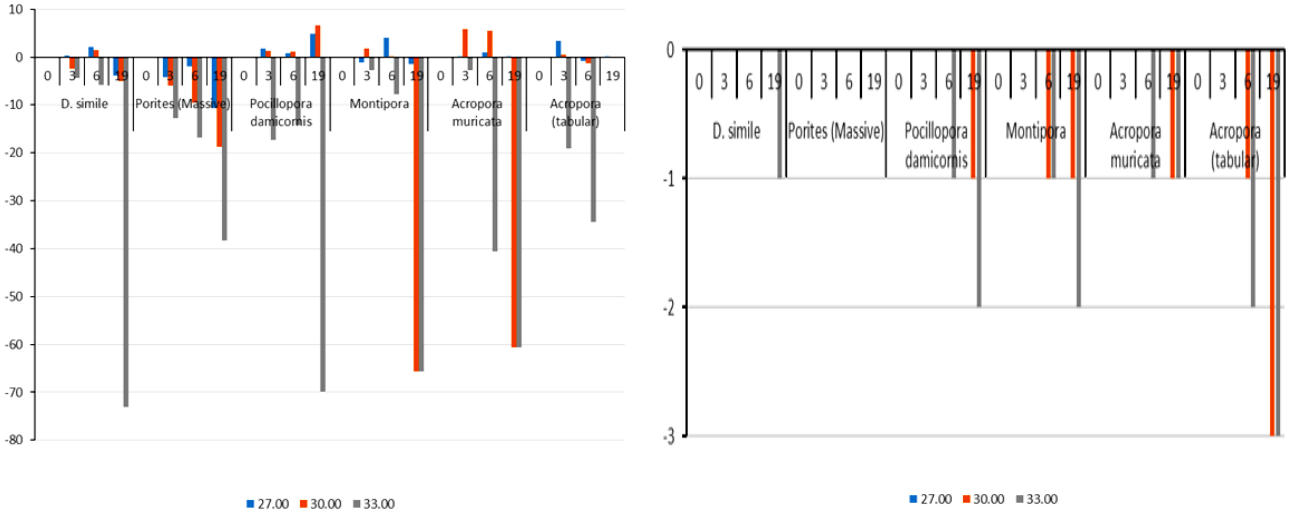
Figure 7. Temperature (A) and light exposure (B) during experimental trials



A **B** **C**

D **E** **F**

Figure 8. Quantum yield at PSII for the ascidian *D. simile* (A), and corals *Porites lutea* (B), *Pocillopora damicornis* (C), *Montipora aequituberculata* (D), *Acropora muricata* (E) and *Acropora cytherea* (F) exposed to temperature trials of 27, 30 and 33ºC at time 0, 3, 6 and 19 hrs of exposure



A **B**

Figure 9. A. Percentage change in mean effective quantum yield at PSII and B. Visual health conditions of treated specimens at 27, 30, and 33ºC at times 0, 3, 6, and 19 hrs. Healthy-looking / Normal: 0; Bleached: -1; Tissue Sloughing: -2; Dead: -3

Discussion

Diplosoma *species identification*

The species' viviparous embedding in a common sheet-like colonial tunic suggested its belonging to the family Didemnidae (Class Ascidiacea; Order Aplousobranchia) (Hirose and Hirose 2013; Goodbody 2000). The further noticed segregated individual arrangement (with zooids on top and embryos at the bottom of the tunic), as well as the presence of 4 rows of stigmata and the lack of atrial aperture, were further features that confirmed its belonging to this genus (Kott 2001; Oka et al. 2005; Stalin et al. 2016). Despite the mention of intra-species polymorphism by Lafargue and Wahl (1987) as being a site-specific adaptive response to the dynamic changes present in the tunicates' environment, which could hinder correct species-level identification (based on morphological features only), accurate identification to genus-level was nonetheless mentioned possible (Lafargue and Wahl 1987; Oka et al. 2005; Koplovitz et al. 2015).

The species, for instance, showed very similar characteristics to the genera *Diplosoma* with its external bright green color and the lack of calcareous spicules mentioned as the most determining features (Kott 1982; Lafargue and Wahl 1987; Hirose and Hirose 2013). The presence of 4 rows of stigmata and the lack of atrial aperture were further features that confirmed its belonging to this genus (Kott 2001; Oka et al. 2005; Hirose and Hirose 2013; Stalin et al. 2016). Despite the high risk of wrong identification triggered by the concept of intra-species polymorphism, the species nonetheless showed further characteristics that seemed to point the direction of identification towards a certain species.

The species stigmata pattern being noticed to follow the arrangement 6-6-6-5 and the presence of a two-lobed testis considerably narrowed down the list of matching species to two, namely: *Diplosoma simile* (Sluiter, 1909) (Sluiter 1909) and *Diplosoma virens* (Hartmeyer, 1909) (Hartmeyer 1909-1911; Koplovitz et al. 2015; Hirose and Nozawa 2010; Goodbody 2000; Oka and Hirose 2008). Despite their outstanding resemblance, authors agreed on some morphological differences, which included the length of the species' embryo, colonies' thickness, and the point of separation of the zooid's retractor muscles (Kott 2001; Hirose and Hirose 2009; Hirose et al. 2009; Hirose and Nozawa 2010; Koplovitz et al. 2015). The *D. virens* is known to have a thick colony (2-5 mm) and harbor a large embryo of approximately 1.5 mm in length, having a small tail (in proportion to the head size) that can wrap only halfway around to the top of the head (Kott 2001; Koplovitz et al. 2015). The *D. simile*, on the other hand, is known to have thinner colonies (1-2 mm) with a smaller embryo (about 800 µm long) with a rather long tail that wraps to the top of its head (Kott 2001). Furthermore, the position where the retractor muscle separates from the thorax tends to be the determining feature between the two species, while in *D. simile*, the muscle emerges directly from underneath the thorax in a T-like shape, in *D. virens*, the muscle separates at a much lower position halfway down the zooid's esophageal neck in an N-like shape (Hirose and Hirose 2009; Hirose et al. 2009). Since the present species' colony was about 1-2 mm thick, had a small embryo (778 µm), which tail wrapped around the top of the head, and had a T-shaped retractor muscle, it is concluded that the studied species is a *D. simile*.

Diplosoma simile *thermal tolerance*

In the last decades, authors agreed on the negative impact of increasing temperatures on marine ecosystems; coral reefs in particular, which are susceptible to undergoing mass bleaching (Bruno et al. 2007; Bhagooli 2009; Barshis et al. 2013; Obura et al. 2017). This phenomenon was proved right after mass bleaching occurred as a result of the ENSO event in 2016, causing up to 60% coral bleaching around Mauritius Island (Obura et al. 2017). The most affected reef-building coral genus being, *Acropora*, which comprised 80% of the coral bleaching, was explained by their low thermal tolerance (~1-2°C higher than normal; in Mauritius being 27-28°C) (Middlebrook et al. 2008; Schoepf et al. 2015). However, the present study showed that *D. simile* has optimal photosynthetic efficiency at temperatures far exceeding the thermal threshold of *Acropora* corals.

Photosymbiotic *Prochloron* sp. (known to be thermo-resistant) was, for instance, recorded to display higher ΦPSII at high temperatures varying between 20°C-40°C when tested in vitro (Dionisio-sese et al. 2001). The reason for the recorded host ascidian bleaching within the optimal temperatures for *Prochloron* sp., however, remains uncertain; either being the result of the stressed *D. simile* colony releasing *Prochloron* sp. in the same way, corals bleach as an adaptive response or due to the photosymbiont itself moving out of the stressed organism. Since the extent of their interdependence is still not fully understood, it is safe not to attribute anything to the reason for ascidian bleaching with rising temperature.

However, the overall liability of the thermal stress experiment in establishing *D. simile* invasiveness remains unclear since it did not encompass the array of factors that affect a species' thermal resistance and hence cannot be used to measure the true extent of the species' thermal tolerance accurately. Time factor certainly was one of them, as indicated in this study. Furthermore, this study did not consider the "environmental condition," "photosymbiotic ultraviolet resistance," or "species' adaptive mechanism" factors that are also known to influence overall fitness (Naranjo et al. 1996; Grimsditch and Salm 2006; Hirose et al. 2004; Pineda et al. 2012; Sensui and Hirose 2018). Bussapakorn et al. (2018) revealed, for instance, the evolution of coral response to thermal stress bringing the multi-dimensional aspect associated with a single species thermo-resistance, the latter being affected by a variety of site-specific conditions (e.g., nutrient load, depth, wave action intensity) as well as regional and international conditions (e.g., ENSO events, earthquake).

The Diplosoma simile *behavior inferred from its distribution pattern*

With the records of mass coral bleaching due to the recent ENSO events, as well as the previous mentions of *D.*

simile characteristics, it can be deduced that the latter species represents a potential threat to Mauritian coral reefs. (Vargas-Ángel et al. 2009; Kremer and Rocha 2011; Hirose and Hirose 2013; Koplovitz et al. 2015; Kaullysing et al. 2016; Obura et al. 2017; Gudka et al. 2018). The notion of invasion, however, also includes an aspect of geographical belonging, whereby a species is unlikely to develop invasive behavior in its native environment (due to ecological integration). Since there is no record of *D. simile* being native to Mauritius, in addition to the records stating its restricted occurrence to shallow waters, it is highly possible that this species was introduced to the Mauritian waters through anthropogenic means (Koplovitz et al. 2015).

The possibility of an introduction by marine transport can be investigated through the species' distribution pattern. It was seen that the highest species occurrence happened in the vicinity of ports or regions with high boat activity (i.e., Balaclava, Flat Island, and Flic en Flac). Furthermore, the region with the lowest species occurrence also seemed to harbor some level of boat activity; Belle Mare and Blue Bay Marine Park are renowned snorkeling spots. An interesting contrast between Balaclava and Blue Bay Marine Parks also seemed to relate to %AC and anthropogenic level. It was further found that among the top 5 ranked beaches in Mauritius, 4 harbors the highest %AC around the Island. Moreover, *D. simile* seemed to occur away from the shore, in regions where most boat activities normally involve recreational purposes. These findings reinforce the probability of an introduction by boat to the Mauritian waters as well as a potential link between the *D. simile* outbreak and the level of anthropogenic activities.

Despite their "higher thermal tolerance," "higher reported resilience," and "resistance to anthropogenic stressors" (contrary to coral communities), the labeling of *D. simile* as an invasive threat to Mauritian reef-building corals remains unclear (Jimenez et al. 2012; Brown et al. 2019). It is to be highlighted that the nature of the reported species' invasive behavior in some countries does not necessarily imply the same pattern to be found in the Mauritian context due to differences in distribution-influencing conditions (Shenkar and Swalla 2011; Koplovitz et al. 2015). Furthermore, the notions of "opportunistic," "invasive," and "dominant" species, often being mixed up in relevant literature, also brought uncertainty to the question, which hence required reference to specific authors in determining *D. simile* behavior. Therefore, since the ascidian was only reported on dead corals, with no means of quantifying its rate of introduction, and since it is still at an early introduction stage (judging by its small population % cover around the Island), the species does not fit Colautti and MacIsaac (2004) description of an invasive species. The *D. simile* seemed to adopt the behavior mentioned by Whitlatch and Zajac (1985) as opportunistic, hence being more apparent to an "opportunistic species" in the Mauritian context.

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Photo-physiology of healthy-looking and diseased/health-compromised hard corals from Mauritius Island, Western Indian Ocean

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Abstract. *Jogee SY, Jeetun S, Ricot M, Taleb-Hossenkhan N, Mattan-Moorgawa S, Kaullysing D, Riemann P, Blanc L, Casareto BE, Suzuki Y, Bhagooli R. 2023. Photo-physiology of healthy-looking and diseased/health-compromised hard corals from Mauritius Island, Western Indian Ocean. Indo Pac J Ocean Life 7: 27-37.* The spatial photo-physiological responses of *in hospite* zooxanthellae in hard corals, including coenosarc and polyps, healthy-looking and affected parts in four coral diseases, namely Brown Band, Black Band, Skeletal Eroding Band and White Band on the coral *Acropora muricata*, and two health-compromised conditions such as the Pink Pigmentation Response and its differentiated morphology, the Pink Line Syndrome, on the coral *Porites* were investigated using the Imaging-PAM fluorometry. A significantly lower F_v/F_m was observed in case of Black Band, White Band, Brown Band and Pink Pigmentation Response affected parts compared to the healthy-looking parts. The F_v/F_m had the highest decline in Brown Band disease. Both the polyps and coenosarc had significantly lower F_v/F_m in White Band and Brown Band diseased parts compared to their healthy-looking parts. The $rETR_{max}$ did not change significantly between diseased/health-comprised parts and healthy-looking parts. NPQ_{max} declined significantly in White Band, Black Band and Pink Pigmentation Response cases. α and β generally did not tend to be affected in diseased/health-compromised conditions. The photo-physiology of *in hospite* zooxanthellae was least affected in Pink Line Syndrome. These findings suggest that diseased/health-compromised parts of corals behave differently in terms of their photo-physiology in different diseased and health-compromised coral conditions in important reef-building corals species such as *A. muricata* and *Porites* species, with important implications for the productivity and thus adaptive management of coral reefs in a globally warming ocean.

Keywords: Black Band, Brown Band, Imaging-PAM fluorometry, polyps, Skeletal Eroding Band, White Band disease

INTRODUCTION

Spatial heterogeneity of photosynthesis in corals and other autotrophic reef-associated organisms is a common phenomenon (Ralph et al. 2002; Hill et al. 2004), with light availability and quantity being strongly altered by the light-matter interactions which can occur at the meso (millimeter to meter) or microscale (micrometer to millimeter) (Anthony and Hoegh-Guldberg 2003). At the mesoscale, spatial heterogeneity occurs between the polyp and coenosarc, which has been observed to display contrasting photosynthetic capacities (Ralph et al. 2002). The light exposure patterns of zooxanthellae can explain the differences in the photosynthetic capacities between the polyp and the coenosarc in these different types of tissues; the zooxanthellae cells inside the coenosarc are constantly exposed to direct and high light illumination, whereas the zooxanthellae cells inside the polyp's endodermal layer are more shade-adapted as they can be retracted at high irradiances (Brown et al. 1994).

The spatial heterogeneity between the polyp and the coenosarc is also apparent under stressful conditions such as thermal bleaching (Hill et al. 2004) or light stress (Ralph et al. 2002). Short-term exposure to high temperatures (33°C) has yielded different photo-physiological responses from the polyp and coenosarc of *Acropora nobilis* and *Cyphastrea serailia* (Hill et al. 2004). The *A. nobilis* also exhibited differential photophysiological responses between its polyp and coenosarc following short-term exposure to high irradiances (1000 µmol photons m^{-2} s^{-1}) (Ralph et al. 2002). Coral diseases and other compromised coral health conditions, such as pigmentation responses, are an example of biotic stressors that can induce physiological alterations in the affected hosts (Rosenberg and Ben-Haim 2002; Roff et al. 2008; Burns et al. 2013). Diseases on marine invertebrates have been on a constant rise over the past several years and are widespread (Harvell et al. 2007), not sparing Mauritian seas (Bhagooli and Klaus 2014; Bhagooli et al. 2017; Mattan-Moorgawa et al. 2017; Bhagooli and Kaullysing 2019; Bhagooli et al. 2021a,b). Given the importance s of Symbiodiniaceae for the subsistence of reef-building corals, it is critical to study the effects of coral diseases on the photo-physiology of affected hosts. The disruption of the coral-algal symbiosis

by coral diseases greatly reduces the productivity of coral reefs since zooxanthellae account for around 50-70% of global benthic reef production (Douglas 2009). By compromising the photo-physiology of the *in-hospite* zooxanthellae, coral diseases inhibit the translocation of photosynthate from the photosynthetic zooxanthellae to the coral host, under healthy-looking conditions satisfies more than 95% of the host nutritional requirements (Muscatine et al. 1984). Coral diseases such as Black Band disease (Roff et al. 2008), White Syndrome (Roff et al. 2008), Skeletal Eroding Band (Roff et al. 2008), White Patch Syndrome, Growth Anomalies (Burns et al. 2013), Brown Band Disease (Ulstrup et al. 2007), and White Band and White Plague (Mattan-Moorgawa et al. 2017) have been reported to compromise the photo-physiology of reef-building corals.

While most of the above studies have assessed the gross spatial heterogeneity in the photo-physiological responses of diseased and health-compromised corals, no studies look at the finer scale differences between the polyp and coenosarc of affected hosts. This study aimed at characterizing and comparing the photo-physiology of healthy and diseased/health-compromised corals and the spatial heterogeneity in chlorophyll fluorescence at the affected hosts' coenosarc and polyp of dominant coral diseases from Mauritius Island, including Skeletal Eroding Band, Brown Band, Black Band, White Band in *Acropora muricata*, and health-compromised conditions like the Pink Pigmentation Response and its differentiated morphology, Pink Line Syndrome in *Porites* species.

MATERIALS AND METHODS

Study site and sample collection

This study was undertaken in the lagoon of Belle Mare, which is located on the east side of the Island of Mauritius (20°11'33.85"S, 57°46'37.04"E) and in the lagoon of Flic en Flac, which is located on the west side of the island (20°16'24.74"S, 57°22'5.14"E) (Figure 1). The depth of the lagoon at Belle-Mare varies from 0-3 m, and the distance from shore to the reef is approximately 850 m (Sadally et al. 2014). Due to heavy precipitation, belle-Mare also experiences algal blooms when fertilizers and sewage washes into the lagoon (Ramessur 2002). In addition, belle-Mare is subjected to higher wind intensity from the South East Trade Winds, as it is located on the Island's east coast. The study area at Belle Mare supported diverse coral assemblages mostly dominated by large branching colonies of *A. muricata*. At Flic en Flac, the lagoon is shallower (0-2 m) and is also dominated by large stands of branching *Acropora* corals in some areas. There is also the presence of underground water seepage at Flic En Flac, which discharges freshwater, nutrients and other land-based chemicals into the lagoon (Ramessur et al. 2011). Coral fragments (n=5 per disease/health-compromised condition) of an affected coral host with different types of lesions (skeletal eroding band, brown band, white band, black band in *A. muricata*, and pink line syndrome and pink pigmentation response in *Porites* sp.) were collected using either a plier or a hammer and a chisel. The lesions were identified on the field using coral disease identification guides such as those in Beeden et al. (2008).

Gross morphological description of coral diseases and compromised coral health conditions

The gross morphology of the coral diseases and compromised health conditions were systematically characterized using the framework proposed by Work and Aeby (2006). This included providing information on the distribution of the lesion (focal, multifocal, multifocal to coalescing, diffuse), the location of the lesion on the colony (central, peripheral, basal, medial, apical), the edges of the lesion (distinct, indistinct), the margins of the lesion (serrated, undulating, smooth, serpiginous), the shape of the lesion (circular, oblong, pyriform, cruciform, linear, lanceolate, irregular), the relief of the lesion (umbonate, bosselated, nodular, exophytic and fimbriated), the color of the lesion, and the texture of the lesion (smooth, rugose). The morphologic diagnosis of the diseases and compromised health conditions was also performed using the framework by Work and Aeby (2006), and additional information on the extent of the lesion (mild (1-20%), moderate (21-50%), severe (51-100%), time (acute, subacute and chronic), lesion (tissue loss, discoloration, growth anomaly) and structures affected (polyp, coenosarc, and skeleton) (Figure 2). Time, herein, refers to the time taken for the development of the disease, and the categories include acute (ranging from hours to days), subacute (weeks to develop), and chronic (months or years to develop).

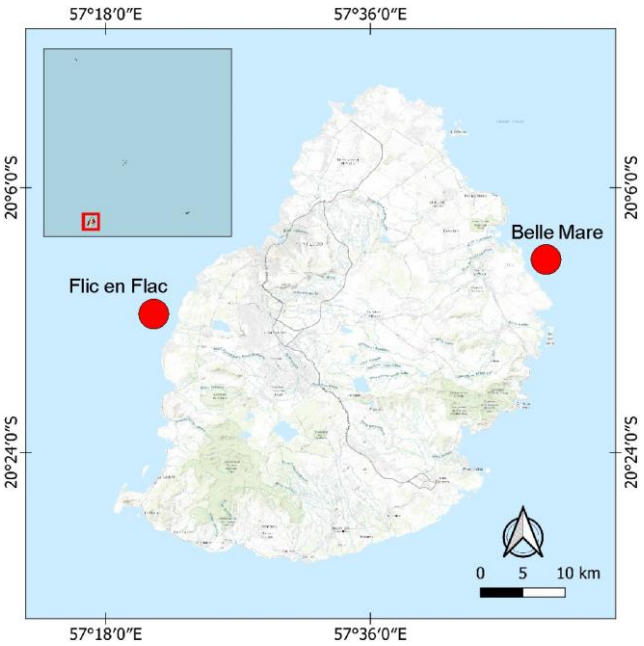


Figure 1. A. Location of Mauritius Island (20^0 34'84'' S, 57^0 55'22''E) in the Indian Ocean, B. Location of Flic en Flac and Belle Mare around Mauritius Island

Figure 2. Examples of (A) Acute disease (Brown Band) with a clear, denuded white skeleton and not overgrown by algae, (B) Sub-acute disease (Skeletal Eroding Band) with denuded skeleton overgrown with filamentous algae and (C) Chronic disease (Pink Pigmentation Response) with no apparent loss of tissue or algal overgrowth

Chlorophyll *a* fluorescence

Chlorophyll *a* fluorescence was measured using an Imaging Pulse-Amplitude-Modulated (I-PAM) fluorometer [Maxi-PAM, M Series, Waltz]. Measurements, through the addition of multiple areas of interest (diameter-5mm), were taken from both diseased and health-compromised and the healthy-looking coral parts. Chlorophyll fluorescence measurements were taken at the lesion and in apparently healthy-looking coral tissues located at least 2 cm from the lesion. By reducing the size of the areas of interest, measurements were also taken on individual polyp and on the coenosarc of close to the lesions and of the healthy-looking coral part. It is noteworthy that three main chlorophyll fluorescence parameters (F_v/F_m, $rETR_{max}$, NPQ_{max}) have been widely used in photo-physiological stress studies in marine organisms (Bhagooli et al. 2021c) and this study focused on these parameters. F_v/F_m represents the maximum quantum yield of Photosystem II (PSII) and it provides and indication of the photosynthetic efficiency of the PSII when all reaction centers are open. $rETR_{max}$ is the maximum relative electron transport rate and it is used to measure the rate of electron transport through the reaction centers in the PSII. NPQ_{max} is the maximum non-photochemical quenching parameter and it gives an indication of the ability of a photosynthetic organism to dissipate excess radiation through non-damaging heat emissions (Bhagooli et al. 2021c).

A double exponential decay function (Platt et al. 1980) was used to fit curves to the Rapid Light Curves (RLCs) and quantitatively compare the parameters such as Alpha (α) (initial slope of the RLC before the onset of saturation), Beta (β) (slope of the RLC after saturation) and $rETR_{max}$ between the healthy-looking and diseased or health-compromised coral parts. $rETR_{max}$, α and β values were obtained after curve fitting through Sigmaplot (Version 12.0).

Statistical analyses

Data were transformed, if necessary, using arcsine-square root transformation to meet the assumptions of normality and equal variance for use of parametric statistical tests. A one-way ANOVA test was used to determine differences (α=0.05) of photo-physiological parameters (F_v/F_m, $rETR_{max}$, NPQ_{max}, α and β) between the diseased or health-compromised lesions and the adjacent healthy-looking parts of the corals. One-way ANOVA was conducted to determine differences (α=0.05) of photo-physiological parameters (F_v/F_m, $rETR_{max}$, NPQ_{max}, α and β) in the polyp and the coenosarc between the healthy-looking and the diseased/health-compromised coral parts. Variations in mean values of photo-physiological parameters (F_v/F_m, $rETR_{max}$, NPQ_{max}, α and β) were analysed, using one-way ANOVA, between the polyp and coenosarc in the disease-affected or health-compromised coral parts.

RESULTS AND DISCUSSION

Morphological description of diseases and health compromised conditions

Four coral diseases (Brown Band, Skeletal Eroding Band, White Band and Black Band) were observed on *A. muricata* and 2 compromised coral health conditions (Pink Pigmentation Response and Pink Line Syndrome) were observed on *Porites* sp. The morphological description of the lesion characteristics of the coral diseases and compromised coral health conditions is summarized in Table 1.

Spatial variation in the photo-physiology of coral diseases

All the coral diseases assessed showed substantial variation in their photosynthetic responses between the healthy-looking part and the diseased lesion (Figure 2). The spatial heterogeneity in the photosynthetic parameters between the healthy-looking part and the diseased part can be observed by the differences in the false-colour images generated by the Imaging-PAM. Within each area of interest chosen, the pixel value of each photosynthetic parameter was automatically averaged and allowed for comparison between the healthy-looking and the diseased coral part. For the Brown Band Disease on *A. muricata*, a 39.3% significant decrease [($F(1, 25) = 252.603$, $p=0.000$] was observed in F_v/F_m in the diseased part compared to the healthy-looking coral part. F_v/F_m was observed to be significantly higher in the polyp [($F(1, 25) = 119.573$, $p= 0.000$]and coenosarc [($F(1, 25) = 124.308$, $p= 0.000$] of the healthy-looking coral part compared to the diseased coral part. For the Skeletal Eroding Band Disease on *A. muricata*, a 13.3% significant decline ($p<0.01$) in NPQ_{max} was observed in the diseased part, compared to the healthy coral part. Significantly lower α ($p<0.01$) was observed in the polyp from the healthy-looking part compared to the diseased part. For White Band disease, a 20.1% and 41.9%

significant decline in F_v/F_m and NPQ_{max}, respectively, was recorded in the diseased coral part compared to the healthy-looking part. F_v/F_m ($p<0.001$) and NPQ_{max} ($p<0.01$) was observed to be significantly higher in the healthy-looking part compared to the diseased part. α was significantly higher in the coenosarc over the White Band-affected coral part compared to the healthy-looking part. However, β was found to be significantly higher in the coenosarc over the healthy-looking part compared to over the diseased part. For the Black Band Disease, a 7.8% significant ($p<0.05$) decrease in F_v/F_m was recorded in the diseased part of the affected coral compared to the healthy-looking part. For NPQ_{max}, a 34.2% significant decrease ($p<0.01$) was observed in the diseased part of the coral compared to the healthy-looking part. F_v/F_m, NPQ_{max} and β was significantly greater ($p<0.01$, Figure 4) in the polyps from the healthy-looking part compared that in the Black Band-affected part.

Spatial variation in the photo-physiology of compromised coral health conditions

The compromised coral health conditions also showed considerable variation in the photosynthetic responses between the health-compromised part and the healthy-looking coral part, as it can be observed from the Imaging-PAM images (Figure 2). For the Pink Pigmentation Response on *Porites lutea*, an 11.6% and a 26.7% significant decline ($p<0.001$) in F_v/F_m and NPQ_{max}, respectively, was recorded in the health-compromised part, compared to the healthy-looking part of the coral. NPQ_{max} was observed to be significantly higher ($p<0.001$) in both the polyp and coenosarc from the healthy-looking part compared to the pigmentation response part. β also was significantly ($p<0.05$) much higher in the coenosarc of the healthy-looking part as opposed to in the Pink Pigmentation Response. No gross scale difference was apparent between the healthy-looking part and the health-compromised part of Pink Line Syndrome, however, slightly lower β was recorded in the coenosarc from the healthy-looking part compared to the Pink Line Syndrome.

Discussion

This study reveals variable photo-physiological responses of diseased/health-compromised coral parts, including comparing the coenosarc and polyps among four coral diseases in *A. muricata* and two health-compromised conditions in *Porites*. In addition, a comparison of photo-physiological effects of diseases on corals from the published literature and this study (Table 2) indicates the varied responses observed.

A pathogenic ciliate causes brown Band Disease from the class Oligohymenophorea, subclass Scuticociliatia (Bourne et al. 2008), which can ingest zooxanthellae cells and harness the photosynthate from the still-photosynthetically competent microalgae (Ulstrup et al. 2007). The significant reduction of Fv/Fm in the diseased coral part compared to the healthy-looking coral part can be explained by the reduction of zooxanthellae density, commonly reported in this disease (Ulstrup et al. 2007). This observation is in contradiction with the findings of Ulstrup et al. (2007) and Roff et al. (2008), whom both recorded no significant difference in Fv/Fm values between the brown band lesion and the healthy-looking coral part of *A. muricata* and *A. nobilis*, respectively (Figure 3). No difference was also observed in the photo-physiological responses of the polyp and coenosarc between the diseased and healthy-looking coral parts. The higher Fv/Fm in the polyp and coenosarc of the healthy-looking part compared to the diseased part can be attributed to the higher zooxanthellae density and higher concentration of photosynthetic pigments in these zooxanthellae cells in both tissue types in that part of the coral (Ulstrup et al. 2007). No difference was observed, however, between the polyp and coenosarc in the Brown Band lesion, and the lack of significant alterations can explain this observation to the coral skeleton and polyp or coenosarc structure affected by the Brown Band Disease and the motility of the ciliates and their ability to migrate across the coral skeleton and to form lesions with varying width and densities which covers both the polyp and coenosarc (Boyett 2006; Lobban et al. 2011). Although differences in the oxygen concentration and Heat Shock Proteins (Hsp) 60 levels have been reported between the healthy part of *A. muricata* and the Brown Band affected part, with relatively lower oxygen concentration and higher Hsp60 levels in the lesion as opposed to the healthy coral part (Ulstrup et al. 2007; Seveso et al. 2015), no fine-scale measurement over the polyp and coenosarc has been recorded so far to be able to explain any spatial heterogeneity in photosynthesis.

The Skeletal Eroding Band is another coral disease caused by the pathogenic ciliate, *Halofolliculina corallasia*. This ciliate is known to disrupt the coral tissues and erodes the coral skeleton of the affected host through the spinning movement of the ciliates and the secretion of chemicals during the formation of the ciliate-housing loricae (Page and Willis 2008). Significant reductions in NPQmax were recorded in the diseased part compared to the healthy-looking coral part of *A. muricata*. Some of this study's observations are in accordance with the findings of Roff et al. (2008), who did not record any significant difference in Fv/Fm or rETRmax of between the healthy-looking and Skeletal Eroding Band-affected parts of *Pocillopora damicornis*. However, a decline in NPQmax was also observed in this study, which contradicts the results of Roff et al. (2008), who did not make such observations. The lack of difference in Fv/Fm can be explained by the presence of photosynthetically competent zooxanthellae, which has also been found to occur in large densities inside the pathogenic ciliates (Cróquer et al. 2006). However, the drop in NPQmax in the diseased coral part might suggest some level of damage to the photosynthetic apparatus of the zooxanthellae. Such damage can be explained by the aggressive movement of the ciliates, which disrupt the coral tissue and *in-hospite* zooxanthellae cells. The higher α value in the diseased part can be explained by the erosion of the coral skeletal structures of the affected coral part, causing potential alterations to the light scattering and diffusion patterns and the availability and efficiency of use of incoming irradiance by the engulfed zooxanthellae. No major difference in the photo-physiological responses of

the polyp and coenosarc was observed across the diseased part. The lack of difference can be explained by the photosynthetically competent zooxanthellae, which are engulfed by the ciliates and which have been observed to embed themselves in both the polyp and coenosarc tissues (Cróquer et al. 2006; Page and Willis 2008). This observation can also be explained by the overgrowth of epiphytic turf algae, which overgrows the dead coral polyp and coenosarc faster than the pathogenic ciliates (Page and Willis 2008), which inhibits the differentiation of the photo-physiological responses between polyp and coenosarc of the affected areas.

White Band Disease is caused by *Vibrio* and *Rickettsiales* bacteria (Ritchie and Smith 1998; Kline and Vollmer 2011). The decline in Fv/Fm and NPQmax in both the polyp and coenosarc of the diseased coral part, compared to the healthy-looking coral part, suggests significant damage to the photosystem of the zooxanthellae in both tissue types. A similar observation, but at a more gross scale, was made by Mattan-Moorgawa et al. (2017), which also reported reduced Fv/Fm and NPQmax in White Band-affected *A. muricata*. The observation made in this study can be explained by the tissue-penetrating action of the pathogenic bacteria associated with this disease. Bacterial penetration inside the tissues of the affected hosts, including both polyp and coenosarc (Croquer et al. 2003), can disrupt the tissue structure and integrity and cause the loss of the *in-hospite* zooxanthellae (Sussman et al. 2009). The putative pathogens of this disease have also been found to have algicidal properties and can cause enzymatic processes (Sunagawa et al. 2009; Silva-Lima et al. 2021), which degrade the coral tissue and cause the loss of the endosymbionts. The bacteria have also been found to inhibit the nitric oxide signaling regulators, which subsequently compromises the coral-algal symbiosis process. All these factors can contribute to causing damage to the photosynthetic function of the zooxanthellae. The lack of difference in the other photo-physiological parameters of the polyp and coenosarc can be associated with the no major structural alteration of the coral skeletal structures and, subsequently, no major changes to the light diffusion and availability patterns in both affected and non-affected coral parts. Higher α in the coenosarc of the diseased part can be explained by the loss of zooxanthellae cells and the higher scattering of light by the less opaque and rugged coral skeleton (Enríquez et al. 2005). Higher β in the coenosarc of the healthy-looking tissues, compared to the diseased part, indicates the presence of photosynthetically competent zooxanthellae in that part which can effectively dissipate the excess irradiances.

A rapidly advancing band characterizes the Black Band Disease, black or deep-reddish brown, separating healthy-looking tissues from the dead and algae-covered skeleton. This black band comprises mostly of a cyanobacterial mat dominated by the *Phormidium corallyticum* species and other groups of sulfate-reducing bacteria, creating an anoxic condition that ultimately kills the coral tissues (Edmunds 1991; Kuta and Richardson 1996; Barneah et al. 2007). The cyanobacteria which constitute the black band are autotrophs which can perform photosynthesis and thus influence chlorophyll fluorescence. Significantly lower Fv/Fm in the Black Band lesion, particularly in the polyps, can be explained by the ability of *P. corallyticum* to create hypoxic conditions for the zooxanthellae occurring near the band (Richardson and Kuta 2003). The observation of this study is to the findings of Roff et al. (2008), who observed significantly reduced Fv/Fm at the interface of the black band lesion and the healthy-looking tissues. Roff et al. (2008) also observed lowered Fv/Fm at the disease interface. Roff et al. (2008) also observed higher NPQmax at the disease interface and hypothesized photoinhibition in the zooxanthellae before the lesion. The lower NPQmax observed at the disease lesion and in the polyps in this study can be explained by considerable damage to the photosynthetic apparatus of the zooxanthellae found close to the lesion. The greater impact on the polyp compared to the coenosarc can be explained by the boring action of the cyanobacteria associated with the Black Band Disease, which has been observed to migrate towards the center of the coral polyp (Miller et al. 2011). The absence of significant differences between the photo-physiological responses between the polyp and coenosarc in the diseased part can be explained by the structure of the cyanobacterial mat, which is commonly associated with the Black Band lesion. The mat's extensive, thick, and diffuse properties hinder the exact estimation and differentiation of the photo-physiological responses between the affected polyp and coenosarc.

Pink Pigmentation Response is one morphological form of Pigmentation Responses and is characterized as brightly colored pink or purple lesions which can be focal or multi-focal or sometimes coalescing on the surface of mostly massive coral colonies. The lesion has been associated with excessive melanin levels inside the coral tissues. Significantly lower Fv/Fm and NPQmax photo-physiological responses were observed in the health-compromised areas compared to the healthy-looking areas of the massive *Porites* coral fragments used in this study. This observation contradicts the findings of Roff et al. (2008), who did not find such significant differences between the affected and non-affected coral parts. The presence of non-fluorescent chromoproteins can explain this difference, but green fluorescent proteins, which have been associated with the Pink Pigmentation Response lesions and are believed to have photo-protective properties for the stressed corals (D'Angelo et al. 2012; Yucharoen 2016). Significant differences were also observed in the NPQmax of the polyp and coenosarc and β in the coenosarc only between the health-compromised and healthy-looking parts of the coral and this can be linked to the morphological changes to the host's skeleton and histological properties. Pink Pigmentation Response lesions are often characterized by thickened corallite walls and loss in coral hardness in the upper margins of the corallites (Yucharoen 2016; Zakaria et al. 2021) with swollen polyps (Thangaradjou et al. 2016), which can influence the light scattering properties over the polyp and coenosarc in the health-compromised part.

Table 1. Morphologic description of the coral diseases or health-compromised coral conditions

Disease name	Species	Distribution of lesions	Location on colony	Edges	Margins	Shapes	Colour	Texture	Extent	Time	Lesion	Structure affected	Mean lesion size (cm)
BBD	*A. muricata*	Diffuse	Central	Annular	Undulating	Circular	Black	Smooth	Severe	Acute	Tissue loss, discoloration	Polyp, coenosarc	0.4±0.1
SEB	*A. muricata*	Diffuse	Medial, basal	Distinct	Smooth	Linear	Grey, black	Smooth	Mild	Acute, sub-acute	Tissue loss	Polyp, coenosar, skeleton	1.8±1.6
WB	*A. muricata*	Diffuse	Medial, basal	Distinct	Smooth	Linear	White	Smooth	Mild	Sub-acute	Tissue loss	Polyp, coenosarc	2.4±1.5
BrB	*A. muricata*	Diffuse	Medial	Distinct	Smooth	Linear	Brown	Smooth	Mild	Acute, sub-acute	Tissue loss, discoloration	Polyp, coenosarc	1.1±0.9
PPR	*P. lutea*	Focal, multifoca, multifocal to coalescing	Colony-wide	Irregular	Undulating	Irregular	Pink, purple	Rough	Mild, moderate	Chronic	Discoloration	Coenosarc	3.6±2.7
PLS	*P. lutea*	Focal, multifoca, multifocal to coalescing	Colony-wide	Irregular, annular	Undulating	Circular, oblong, irregular	Pink	Rough	Mild, moderate	Chronic	Tissue loss, discoloration	Polyp, coenosarc	0.2±0.3

Note: BBD: Brown Band Disease, SEB: Skeletal Eroding Band; WB: White Band; BrB: Black Band; PPR: Pink Pigmentation Response: PLS: Pink Line Syndrome

Table 2. Summary of the photo-physiological impacts of coral diseases and health-compromised conditions, compared to the findings of this study

Coral disease	Coral species	Location	Photo-physiological impact in diseased/health-compromised part compared to healthy-looking part	Sources
Brown Band Disease	*Acropora muricata*	Davies Reef, Great Barrier Reef, Australia	Higher F_v/F_m, Lower α	Ulstrup et al. (2007)
		Mauritius Island	Lower F_v/F_m	This study
	A. nobilis	Heron Reef, Great Barrier Reef, Australia	Lower ETR_{max}, Lower α	Roff et al. (2008)
Skeletal Eroding Band	*Pocillopora damicornis*	Heron Reef, Great Barrier Reef, Australia	No significant change	Roff et al. (2008)
	A. muricata	Mauritius Island	Lower NPQ_{max}	This study
White Band	*A. muricata*	Mauritius Island	Lower F_v/F_m, $rETR_{max}$ and NPQ	Mattan-Moorgawa et al. (2017)
			Lower F_v/F_m and NPQ_{max}	This study
Black Band	*Cyphastrea microphthalma*	Heron Reef, Great Barrier Reef, Australia	Reduced F_v/F_m, Higher NPQ_{max}	Roff et al. (2008)
	A. muricata	Mauritius Island	Lower F_v/F_m, Lower NPQ_{max}	This study
Pink Pigmentation Response	*Porites* species	Heron Reef, Great Barrier Reef, Australia	No significant change	Roff et al. (2008)
		Mauritius Island	Lower F_v/F_m, Lower NPQ_{max}	This study
Pink Line Syndrome	*Porites* species	Mauritius Island	No significant change	This study

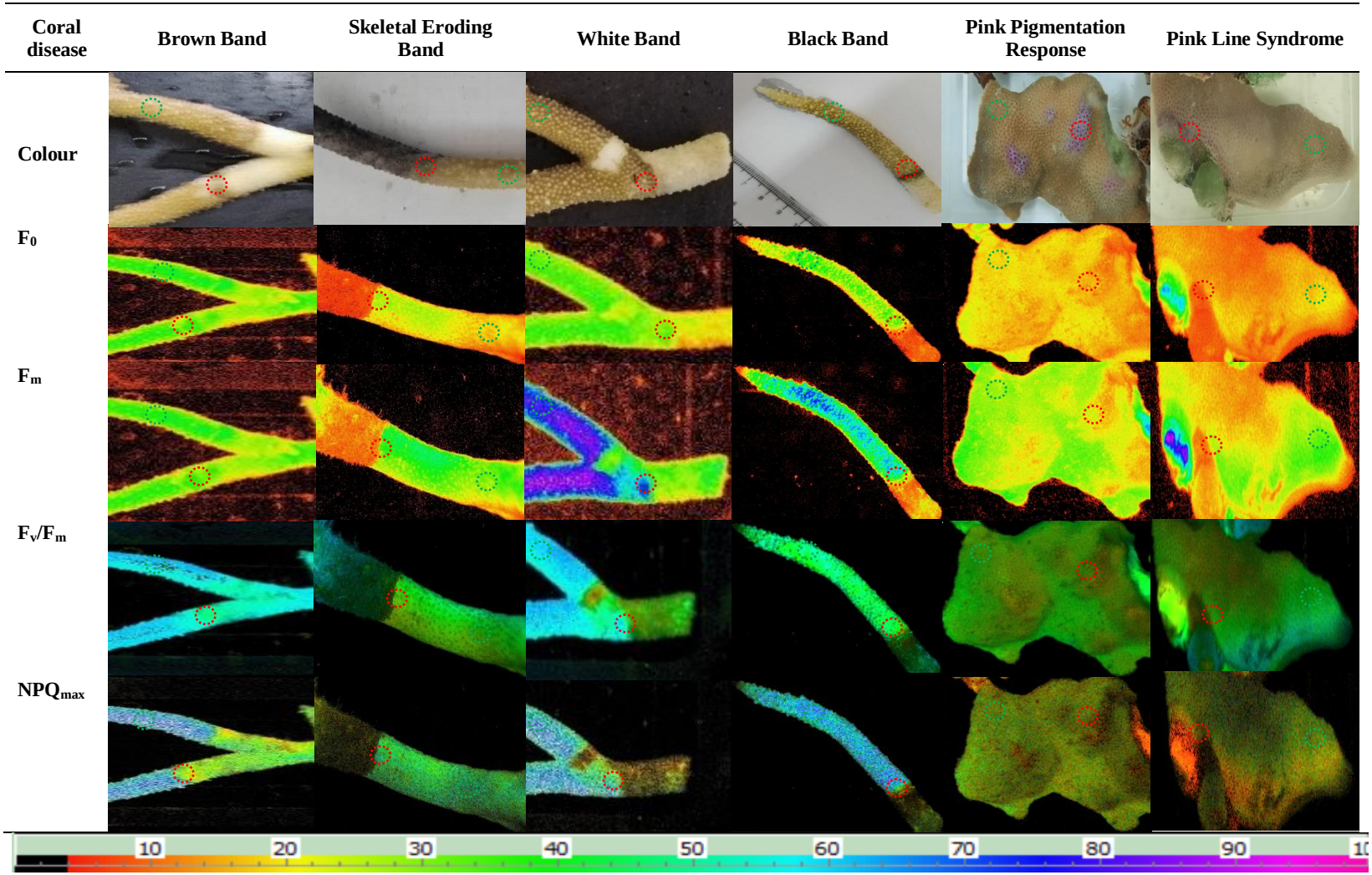
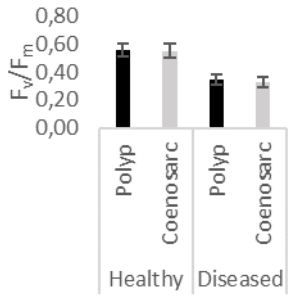
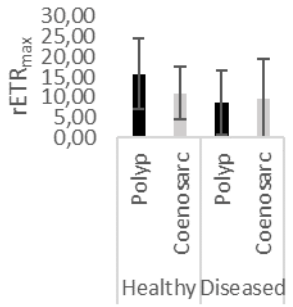
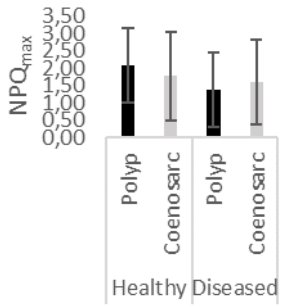
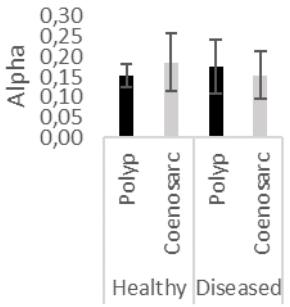
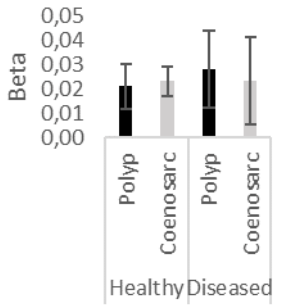
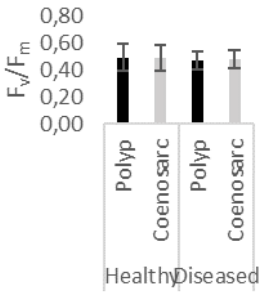
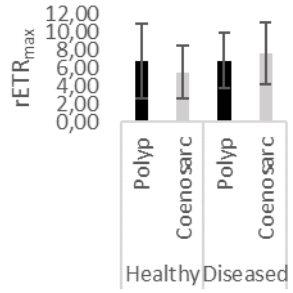
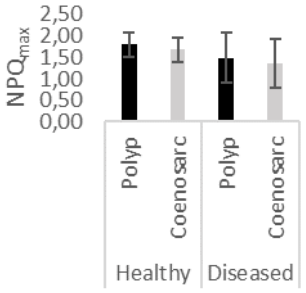
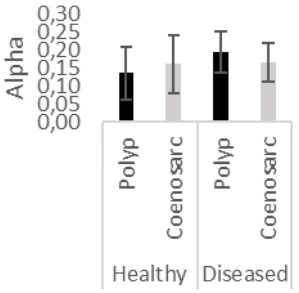
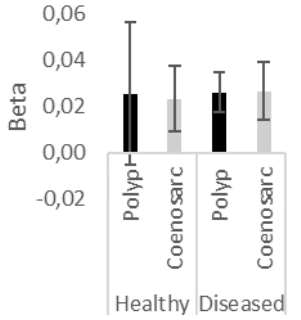
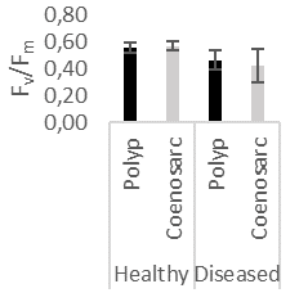
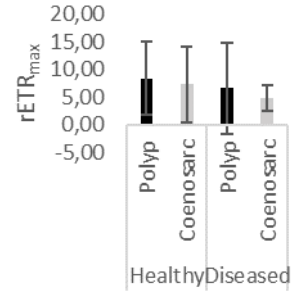
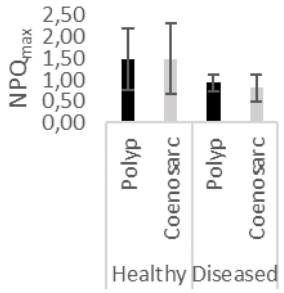
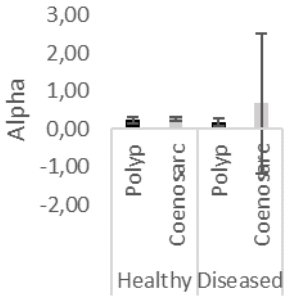
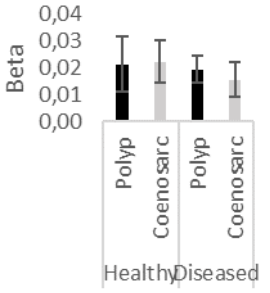


Figure 3. Colour, F_0, F_m, F_v/F_m, NPQ_{max} images of brown band, skeletal eroding band, white band, black band and pink pigmentation response, pink line syndrome (Red circles indicate measurement point for diseased or health-compromised part and green circle indicate measurement point for healthy-looking part). The false colour scale shows the relative values ranging from 0 to 1

Disease/compromised health condition	Maximum Quantum Yield (Fv/Fm)	Maximum Relative Electron Transport Rate (rETRmax)	Maximum Non-Photochemical Quenching (NPQmax)	Alpha (α)	Beta (β)
Brown Band Disease					
Skeletal Eroding Band Disease					
White Band Disease					

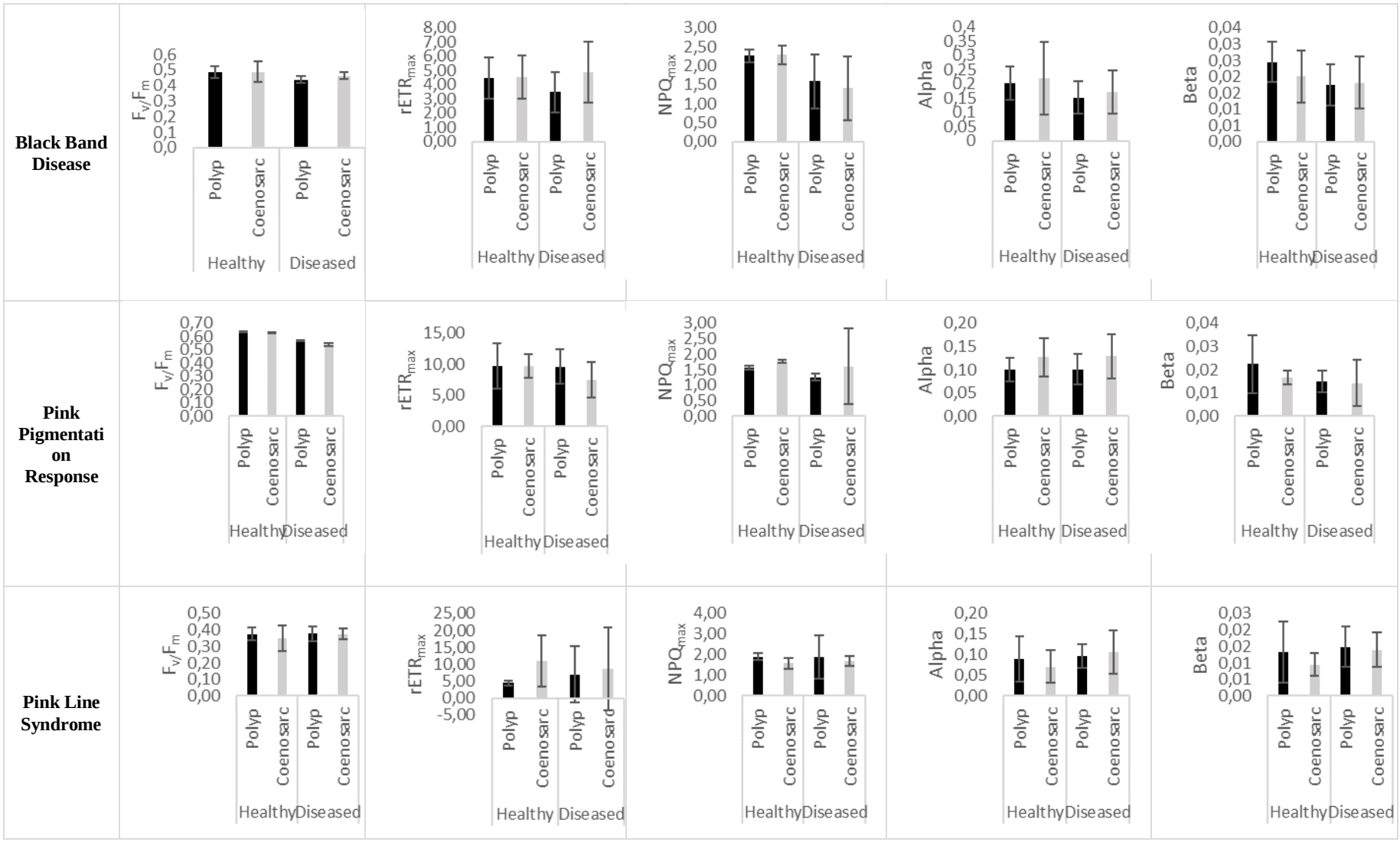


Figure 4. F_v/F_m, $rETR_{max}$, NPQ_{max}, α and β of the polyp and coenosarc of diseased-affected and health-compromised and healthy-looking coral part. Bars represent mean ± SD

The Pink Line Syndrome, a differentiated morphology of Pink Pigmentation Response, is characterized by a brightly pigmented and inflamed pink-colored line separating healthy-looking coral tissues from the dead and algae-covered dead coral skeleton. Pink Line Syndrome has been associated with a cyanobacterium, *Phormidium valderianum*, penetrating the coral tissues and disrupting their integrity (Ravindran and Raghukumar 2002). However, in this study, no significant difference in the photo-physiological responses was observed between the healthy and health-compromised coral parts, similar to another study by Roff et al. (2008), who also did not find any photo-physiological differences between pigmentation responses and the healthy-looking coral part.

The findings of this study show differential spatial heterogeneity in the gross (between the healthy-looking part and the diseased/health-compromised part) and fine-scale (between the polyps and coenosarc) photo-physiological responses among different coral diseases and compromised health conditions on dominant reef-builders found around the island of Mauritius. The findings of this study will have serious implications for the mitigation and management of coral diseases and compromised coral health conditions, given the importance of the photo-physiology of reef-building corals as a critical and proactive indicator of coral health. However, with global climate change, further research is warranted to assess the fine- and gross-scale temporal changes related to the spatial heterogeneity in the photophysiological responses of coral diseases and health-compromised conditions.

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Thermal photo-physiological responses of massive heat-resistant coral *Porites lutea* under fish predated versus non-predated conditions

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Abstract. *Ricot M, Jeetun S, Joggee S, Kaullysing D, Taleb-Hossenkhan N, Bhagooli R. 2023. Thermal photo-physiological responses of massive heat-resistant coral* Porites lutea *under fish predated versus non-predated conditions. Indo Pac J Ocean Life 7: 38-47.* Fish predation on corals leading to polyp and tissue loss has been identified as a significant stressor to corals and is often associated with reduced growth, reproduction, and even mortality. However, how climate change-driven ocean warming may impact such a biological stressor is yet to be thoroughly understood. This study aimed to assess elevated temperature's effects on the photo-physiology of fish-predated and non-predated parts of the thermally resistant coral *Porites lutea* (Quoy & Gaimard, 1833). The objectives were to assess the photo-physiological parameters such as effective quantum yield at photosystem II (FPSII), relative maximum electron transport rate ($rETR_{max}$), maximum photo-chemical quenching (NPQ_{max}), photosynthetic efficiency (α), photoinhibition (β) and Ik at fish bite-affected (BA) and non-affected (NA) coral parts at temperatures of 28°C and 32°C under low-light (10 µmol quanta $m^{-2}s^{-1}$) and moderate-light (110 µmol quanta $m^{-2}s^{-1}$) conditions for a duration of 48hr. FPSII, $rETR_{max}$ and NPQ_{max} were not negatively affected by fish predation conditions. Under moderate light, the $rETR_{max}$ increased in the non-predated condition at 28°C but not at 32°C while the NPQ_{max} exhibited a more pronounced increase at 32°C compared to the 28°C treatment. The absence of significant declines in FPSII and $rETR_{max}$ accompanied by a significant increase in NPQ_{max} at 32°C is indicative of a lack of photo-inhibition and an active quenching of energy in a non-harmful way at PSII. No significant interactions of temperature and predation condition and light and predation condition were found, indicating that short-term exposure of 2 days to an elevated temperature of 32°C and moderate light intensity of 110 µmol quanta $m^{-2}s^{-1}$ did not result in any exacerbated negative photo-physiological impacts of fish predation in *P. lutea*. These findings suggest that both fish-predated and non-predated conditions in *P. lutea* are equally tolerant to the tested elevated temperature level. Thus, ocean warming events may not differentially impact their photosynthetic activities.

Keywords: Elevated temperature, photo-physiology, *Porites*, predation, stress

INTRODUCTION

Coral reefs support an array of ecosystem goods and services that contribute to the well-being of millions of people (Woodhead et al. 2019). However, the coral reefs have endured disturbances of such broad scale and severity in the past decade, that changes in the coral assemblages, and composition and physical appearance were observed (Hughes et al. 2017, 2018). Coral reefs throughout the tropics have been severely impacted by climate change through tropical storms and coral bleaching (Hughes et al. 2018). Extensive loss of coral cover owing to coral mortality following temperature-induced bleaching has been reported globally (Monroe et al. 2018; Sully et al. 2019) and around Mauritius (Bhagooli and Klaus 2014; Bhagooli and Kaullysing 2019; Bhagooli et al. 2021a; McClanahan and Muthiga 2021). Moreover, the uneven susceptibility of corals of different taxa to stressors has led to broad changes in the coral community (Huertas et al. 2021). The species such as the massive *Porites*, which are bleaching-resistant, often survive bleaching episodes that are capable of killing other corals which are more susceptible (McClanahan et al. 2007; Prachett et al. 2013). For instance, on the Great Barrier Reef, a rise in the proportion of massive *Porites* compared with the remaining coral cover has been reported (Hughes et al. 2018).

The changes in coral cover resulting from acute disturbances are rapidly noticeable (Hughes et al. 2018) and aftermaths of coral loss has domino effects throughout the whole ecosystem (Prachett et al. 2011). Hence, the probable loss of biodiversity in these systems has put forward the crucial need to comprehend the main factors sustaining the diversity of the reef and upholding coral reef resilience (Bellwood et al. 2004), which is important as the effect of climate change on coral reef is expected to escalate (Bonaldo and Bellwood 2011; Hughes et al. 2017). In this line, coral predation by reef fishes has been investigated (Rotjan and Lewis 2008; Mumby 2009; Rotjan and Lewis 2009), exhibiting the significance of predation as one of the major natural factors influencing the distribution and abundance of corals on coral reefs (Littler et al. 1989; Rotjan and Lewis 2005).

Even though parrotfishes mostly feed on substratum covered by algal turf, they scrape the surface of live corals as well (Rotjan and Lewis 2005; Bonaldo and Bellwood 2011; Bruckner and Bruckner 2015). Under particular

conditions, parrotfish corallivory may compromise the coral survival and henceforth regulate their abundances and distribution (Rotjan and Lewis 2005; Rotjan et al. 2006; Mumby 2009; Bonaldo and Bellwood 2011).

Colonies of hermatypic corals have proven to be very sensitive to thermal stress (Marshall and Baird 2000; Hoegh-Guldberg 2011) and the magnitude of predation can cause further ripple-effects on the corals (Kayal et al. 2012). Lacerations to tissues and wounds to the aragonite skeleton caused by predation require substantial energetic, molecular and cellular supplies in order to restore (Rice et al. 2019). Even small injuries on the skeletal or tissue structures can induce disturbances in growth and cause fitness consequences (Rotjan and Lewis 2008). Additionally, recurrent corallivory can become a source of chronic stress (Madeira et al. 2022).

Eco-physiological studies brought about insights into mechanisms used by corals while coping through thermal stress challenges (Madeira et al. 2022). Chlorophyll a fluorescence is being employed to a greater extent as a fast, non-intrusive, accurate, and practical measure of photosynthetic activity in marine photosynthetic organisms. This method has been used extensively in research to identify stress, resistance and acclimation/adaptation to stress in marine autotrophic organisms, particularly in the context of a globally changing marine climate (Bhagooli et al. 2021b)

As coral reefs cope with warming oceans and other anthropogenic stress, it is important to understand how these stressors will impact important interactions such as coral predation and their influence on the ecosystem structure and function of the coral reef. Therefore, this study aimed at investigating the short-term combined effects of elevated sea temperature and predation on massive *P. lutea*. We assessed the photo-physiological parameters of coral parts affected by fish bites (BA) and those that were not affected (NA) at temperatures of 28°C and 32°C under low-light (10 µmol quanta $m^{-2}s^{-1}$) and moderate-light (110 µmol quanta $m^{-2}s^{-1}$) conditions.

MATERIALS AND METHODS

Collection sites and sample collection

Collection of massive *Porites* samples were carried out at the backreef at Albion (20.2081°S, 57.4090°E), which is located on the west coast of Mauritius Island (Figure 1). The site was selected according to the ease of access and high occurrence of massive *Porites*.

Porites lutea (Quoy & Gaimard, 1833) was identified in situ according to Veron (2000). The *P. lutea* with visible parrotfish scars were identified as per the description in Benaldo et al. (2011) and were sampled (Figure 2). A total of 12 fragments (4 from each of the 3 colonies) of *P. lutea* having both fish bite-affected (BA) and non-affected (NA) parts were collected by using a hammer and chisel during snorkelling. The coral samples were transported in an isotherm box and kept under shade to the laboratory for experimentation.

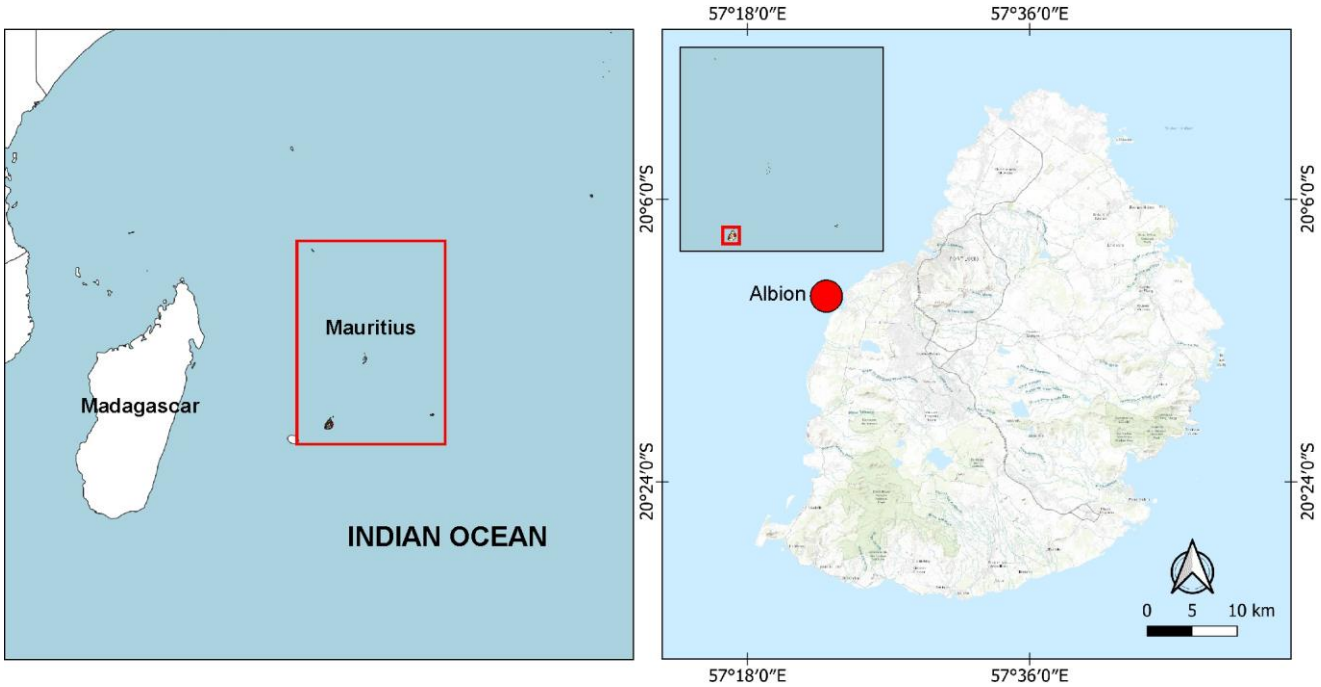

Figure 1. Location of Mauritius Island in the Indian Ocean (*left*). Map showing Albion, the collection site (*right*)

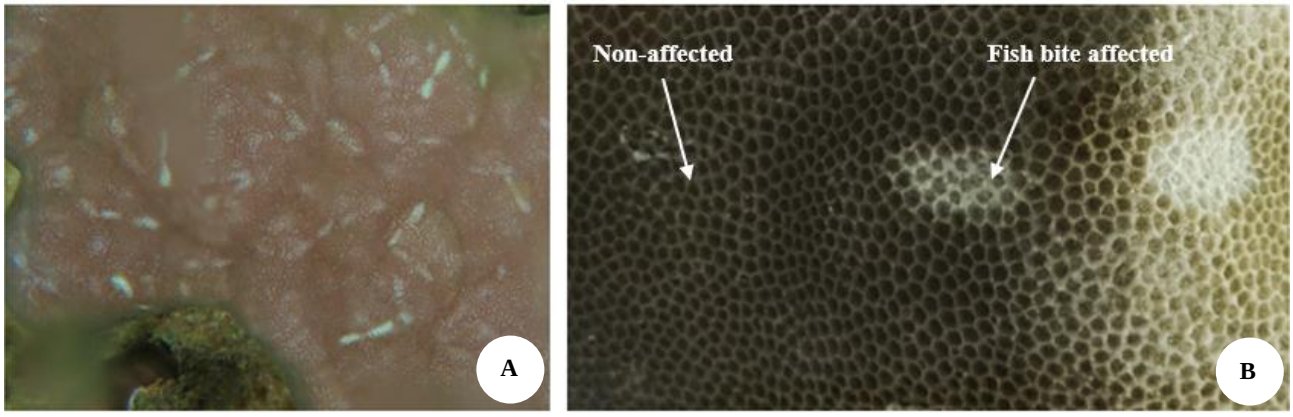

Figure 2. The *P. lutea* in-situ. A. Fish predation scars; B. Close-up of non-affected and fish-bite affected coral parts

Experimental design and procedures

Coral samples were exposed to temperatures ramped up within 2 hr to 28°C and 32°C from 6 a.m. to noon and allowed to recover at 27°C in the afternoon, in order to mimic field-like daily thermal variations. The samples were also exposed to two light conditions (approximately 110 µmol quanta $m^{-2}s^{-1}$ and 10 µmol quanta $m^{-2}s^{-1}$) for 12 hr, followed by 12 hr of no light (dark). The Hobo pendant data loggers were used to track the changes in temperature. The Chlorophyll *a* fluorescence was measured at both the non-affected and the fish-bite affected coral parts whereby the measurement of chlorophyll *a* fluorescence, using the Imaging Pulse Amplitude Modulated fluorometer (Model: IMAG-K7) (Bhagooli et al. 2021b), commence by the switching on a weak measuring light (<1 µmol quanta $m^{-2}s^{-1}$) that yields a measure of the minimal level of fluorescence (F_0) (Figure 3). A saturating white light flash is then applied to the coral parts to obtain the measurement of F_m, the maximum fluorescence value, when all the photosynthetic reaction centres are closed. Hence, the maximum quantum yield at PSII (F_v/F_m) for the BA and NA parts were recorded using a diving Pulse-Amplitude-Modulated fluorometer (D-PAM) before the exposure to temperatures of 28°C and 32°C and during subsequent recovery period (evening). The effective quantum yield at PSII was recorded just before noon; when exposure to temperature was expected to be the highest, and just after noon; at the beginning of the recovery period, using D-PAM and Imaging Pulse-Amplitude-Modulated fluorometer (I-PAM), respectively. The relative electron transport rate (rETR) and the non-photochemical quenching (NPQ) were also determined through the rapid light curves (RLCs) using I-PAM. During the rapid light curve, the corals were illuminated from 0 to 1325 µmol photon $m^{-2}s^{-1}$ in 8 phases of 10 seconds each. A double exponential decay function (Platt et al. 1980) was used to fit curves to the RLCs and quantitatively compare descriptive parameters, namely $rETR_{max}$, the onset of saturation (α), the onset photo-inhibition (ß) and the minimum saturating irradiance (I_k). The photosynthetic parameters were calculated as follows:

Effective Quantum Yield (ϕ PSII) = $(F'_m - F_t)/F'_m$, where F'_m is the light-adapted maximum fluorescence and F_t is the fluorescence before a saturating pulse (Genty et al. 1989).

Maximum Quantum Yield (F_v/F_m) = $(F_m - F_o)/F_m$

Non-Photochemical Quenching (NPQ) = $(F_o - F'_m)/F'_m$

Relative Electron Transport Rate (rETR) = ϕ PSII x PAR x 0.5, where 0.5 is the factor for light partitioning between PSII and PSI.

$I_k = {}_rETR_{max}/ ß$

Statistical analyses

The absolute values were arsine-square root transformed where necessary to meet the assumptions of normality and equal variances for the use of parametric statistical tests. A three-way ANOVA test was used to test for differences in the photo-physiological parameters (F_v/F_m, $_rETR_{max}$, NPQ_{max}, α, ß, and I_k) in the NA and BA parts of the coral samples exposed to temperatures of 28°C and 32°C under light intensities of 10 µmol quanta m^{-2} s^{-1} and 110 µmol quanta m^{-2} s^{-1}.

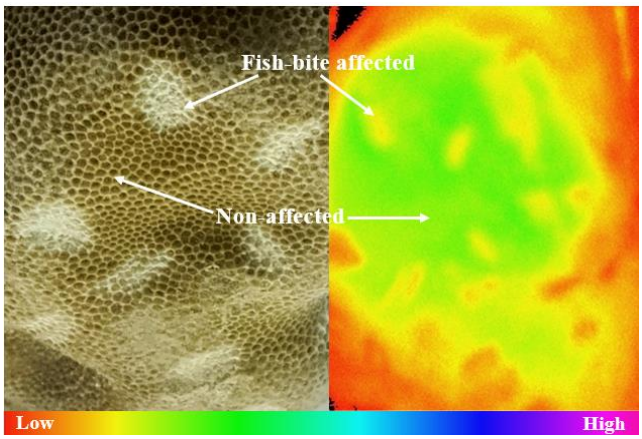

Figure 3. Fluorescence (F_o) at fish-predated and non-predated areas on *P. lutea* as seen normally (*left*) and using the Imaging-PAM fluorometer (*right*)

RESULTS AND DISCUSSION

Temperature and light intensity variations during the experimental trial

The temperature recorded using the data loggers during the experimental trials indicated that the tanks were maintained at 28°C or so during the day and 24 to 25°C during the night in tanks labeled 28°C and at 32°C or so during the day and 25 to 26°C during the night in tanks labeled 32°C (Figure 4 and Table 1). Light conditions were maintained at approximately 110 µmol quanta $m^{-2}s^{-1}$ of ML and 10 µmol quanta $m^{-2}s^{-1}$ for LL labeled tanks for 12 hr, followed by 12 hr of no light (dark).

Photo-physiological responses of *Porites lutea*

The three-way ANOVA (Table 2) revealed that there was no negative effect on all the measured photo-physiological responses of the *P. lutea* irrespective of bite-affected or non-affected conditions. Additionally, the maximum electron transport rate and the non-photochemical quenching were found to be also affected by the light (ETR_{max}, P = 0.031 and NPQ, P = 0.011) and temperature (ETR_{max} and NPQ, $P < 0.001$) conditions. α, ß and I_K were significantly affected by the light.

At the low light treatment, the effective quantum yield for the BA and NA parts exposed to 28°C increased (p<0.05) from 0.45±0.01 to 0.53±0.03 and, 0.50±0.01 to 0.63±0.04, respectively (Figure 5). However, following the 48hr treatment the corals exposed to 32°C conditions, showed almost no change in the effective quantum yield, both for the bite-affected and non-affected parts. Additionally, at the moderate light treatment, the effective quantum yield of the BA and NA parts exposed to 28°C increased by approximately 12% (p<0.05) from 0.47±0.03 to 0.52±0.04 and, 0.48±0.05 to 0.61±0.01, respectively, while at the 32°C treatment, a minor decrease was noted for the bite-affected part. However, no significant difference was found in the effective quantum yield for the coral parts exposed at 32°C.

An increase of 15%, however not significant, was noted in the maximum electron transport rate of the coral parts exposed to the low light treatment, except for the BA exposed at 32°C which remained almost unchanged (Figure 6). At the moderate light treatment, the $rETR_{max}$ recorded for the BA and NA parts, both 28°C and 32°C increased by approximately 20%. However, only the NA parts showed significant increase in $rETR_{max}$ (P<0.05). The highest increased in the maximum electron transport rate was observed for the NA parts at 32°C temperature treatment (10.13±2.98 vs 20.13±1.04).

Moreover, the NPQ of the BA and NA parts showed a trend of elevation for both temperature conditions, at both the low light and moderate light treatments (Figure 7). However, the increase in NPQ was not significant, except for the NA part exposed at 28°C. The highest increase in the NPQ (25%) was observed for the moderate light treatment for the BA parts at 32°C.

A significant increase in α (p<0.05) was noted for the NA parts exposed to low light at 28°C and, the BA parts exposed to moderate light at 28°C (Figure 8) However the corals exposed to 28°C and 32°C under both low light and moderate light conditions, exhibited no significant change in ß (Figure 9) and I_k (Figure 10).

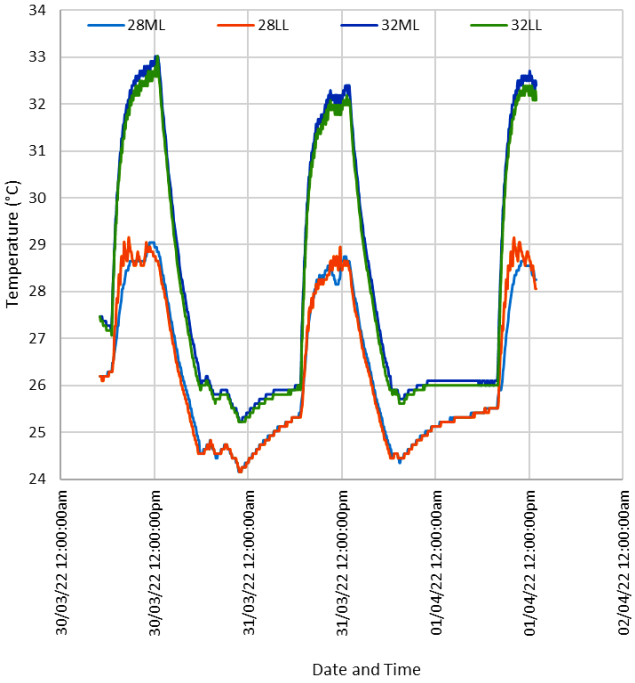

Figure 4. Temperature (°C) variation during the experimental trial. The different coloured lines represent different treatment (light blue: moderate light -28°C (28ML), orange: low light -28°C (28LL), dark blue: moderate light -32°C (32ML) and, green: low light -32°C (32LL))

Table 1. Summary of thermal exposures during the day following the ramping up of temperature within 2hr and night time experimental trials

Day	Time of the day	Temperature and light conditions			
		28LL: 28°C + 10 µmol quanta m^{-2} s^{-1}	28ML: 28°C + 110 µmol quanta m^{-2} s^{-1}	32LL: 32°C + 10 µmol quanta m^{-2} s^{-1}	32ML: 32°C + 110 µmol quanta m^{-2} s^{-1}
1	Day time	28.77 ± 0.17	28.71 ± 0.18	32.65 ± 0.24	32.41 ± 0.26
	Night time	24.53 ± 0.17	24.53 ± 0.17	25.67 ± 0.26	25.60 ± 0.25
2	Day time	28.48 ± 0.21	28.86 ± 0.09	32.18 ± 0.13	32.00 ± 0.11
	Night time	25.13 ± 0.33	25.11 ± 0.33	26.03 ± 0.11	25.94 ± 0.11
3	Day time	28.50 ± 0.12	28.67 ± 0.26	32.47 ± 0.12	32.22 ± 0.12

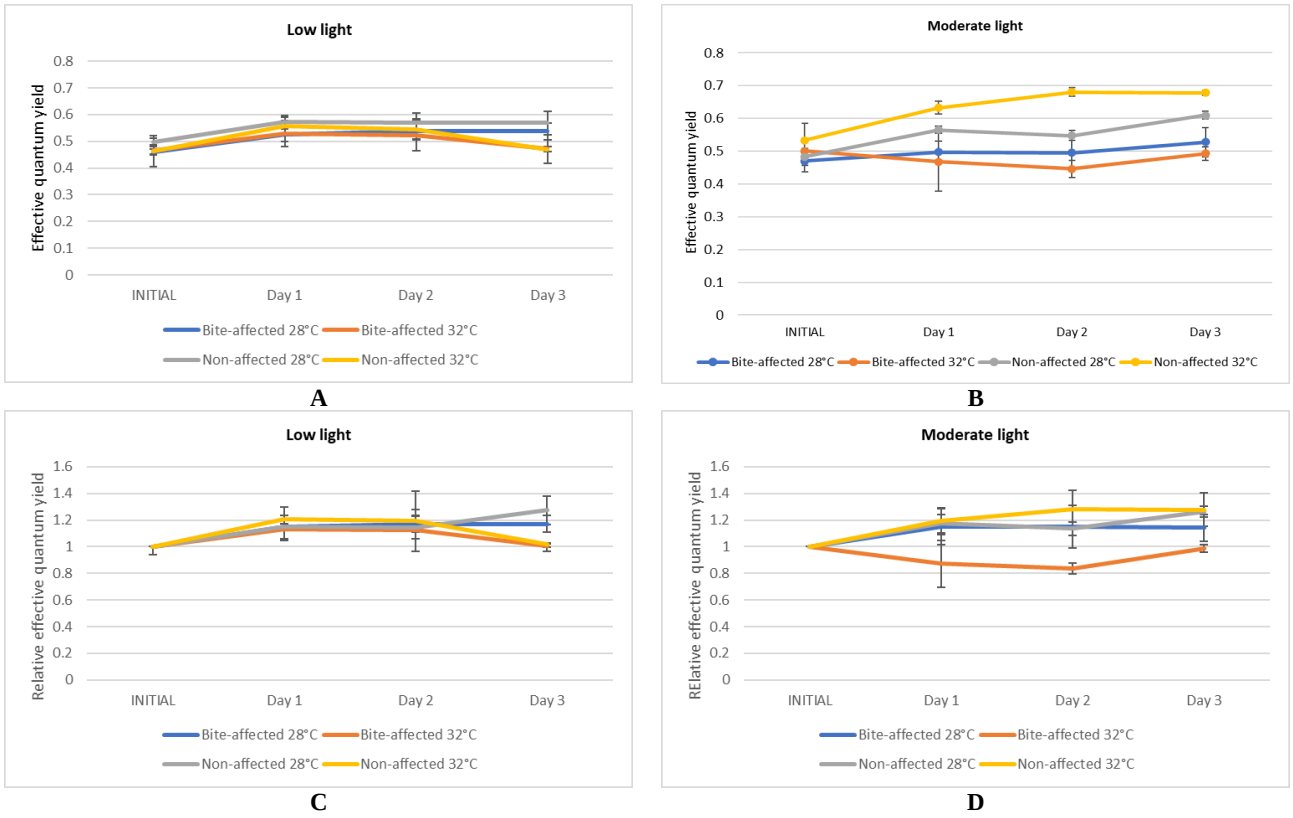

Figure 5. Effective Quantum Yield of BA and NA parts at 28°C and 32°C at: A. Low light treatment, B. Moderate light treatment, and the relative effective quantum yield of the BA and NA parts at 28°C and 32°C at: C. Low light treatment; D. Moderate light treatment. Data represents mean ± SD (n=3)

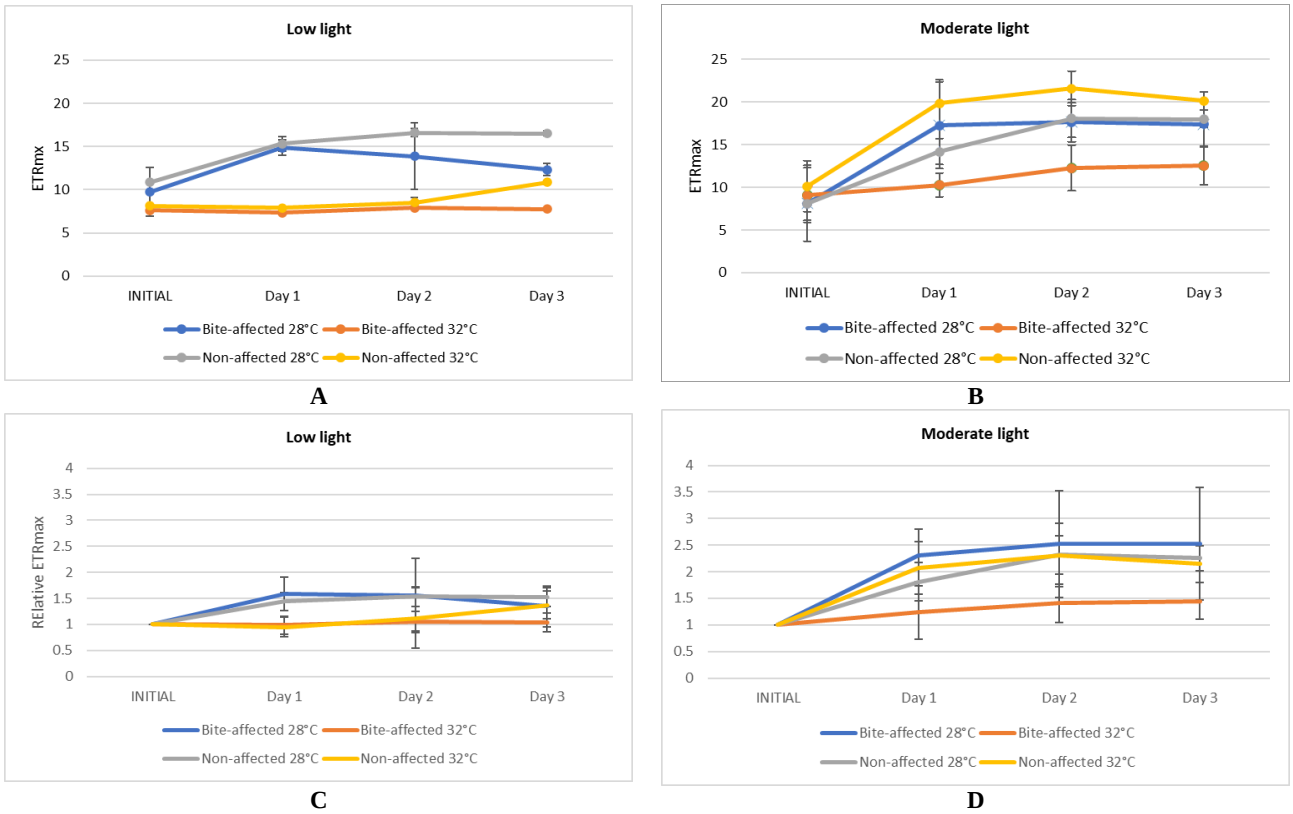

Figure 6. Maximum electron transport rate ($rETR_{max}$) of BA and NA parts at 28°C and 32°C at: A. Low light treatment, B. Moderate light treatment, and the relative maximum electron transport rate of the BA and NA parts at 28°C and 32°C at: C. Low light treatment; D. Moderate light treatment. Data represents mean ± SD (n=3)

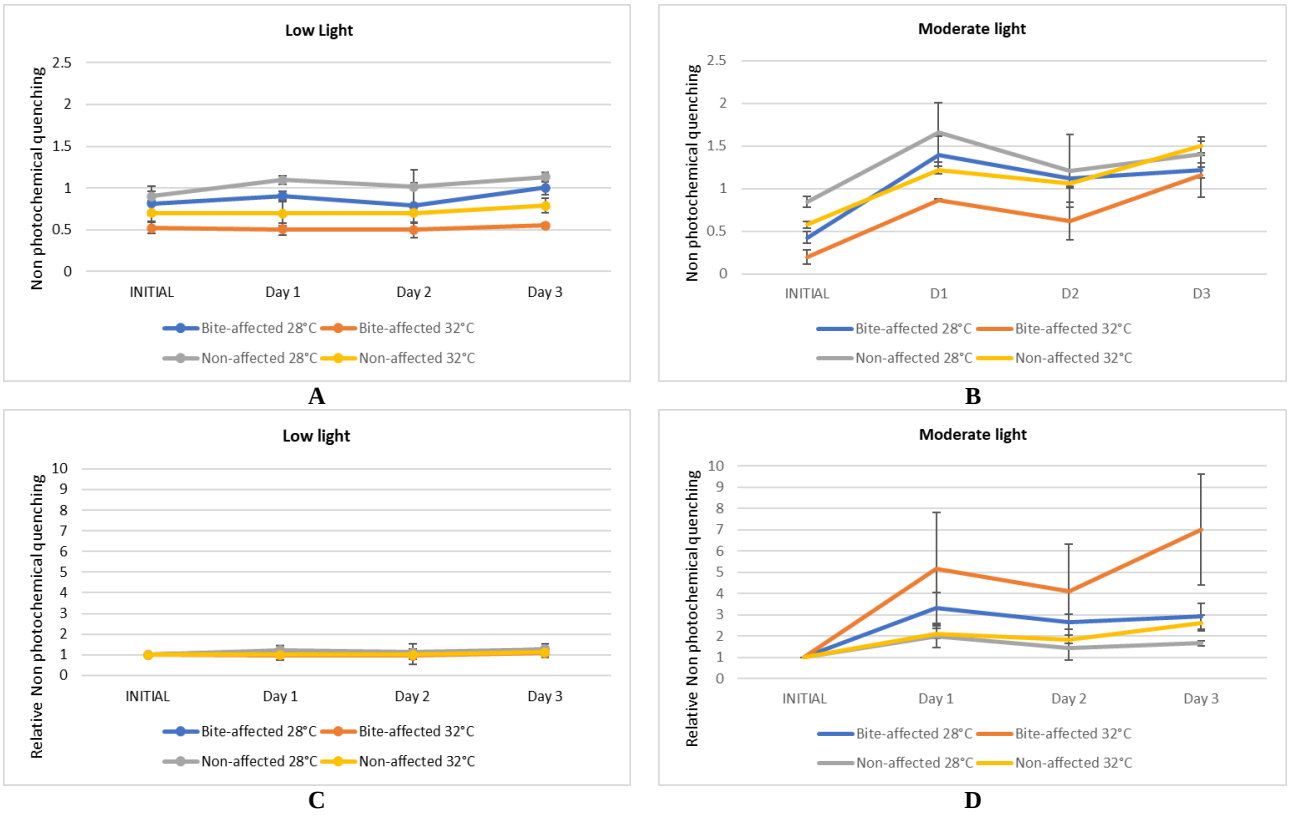

Figure 7. Non photochemical quenching (NPQ) of BA and NA parts at 28°C and 32°C at: A. Low light treatment; B. Moderate light treatment, and the relative NPQ of the BA and NA parts at 28°C and 32°C at: C. Low light treatment; D. Moderate light treatment. Data represents mean ± SD (n=3)

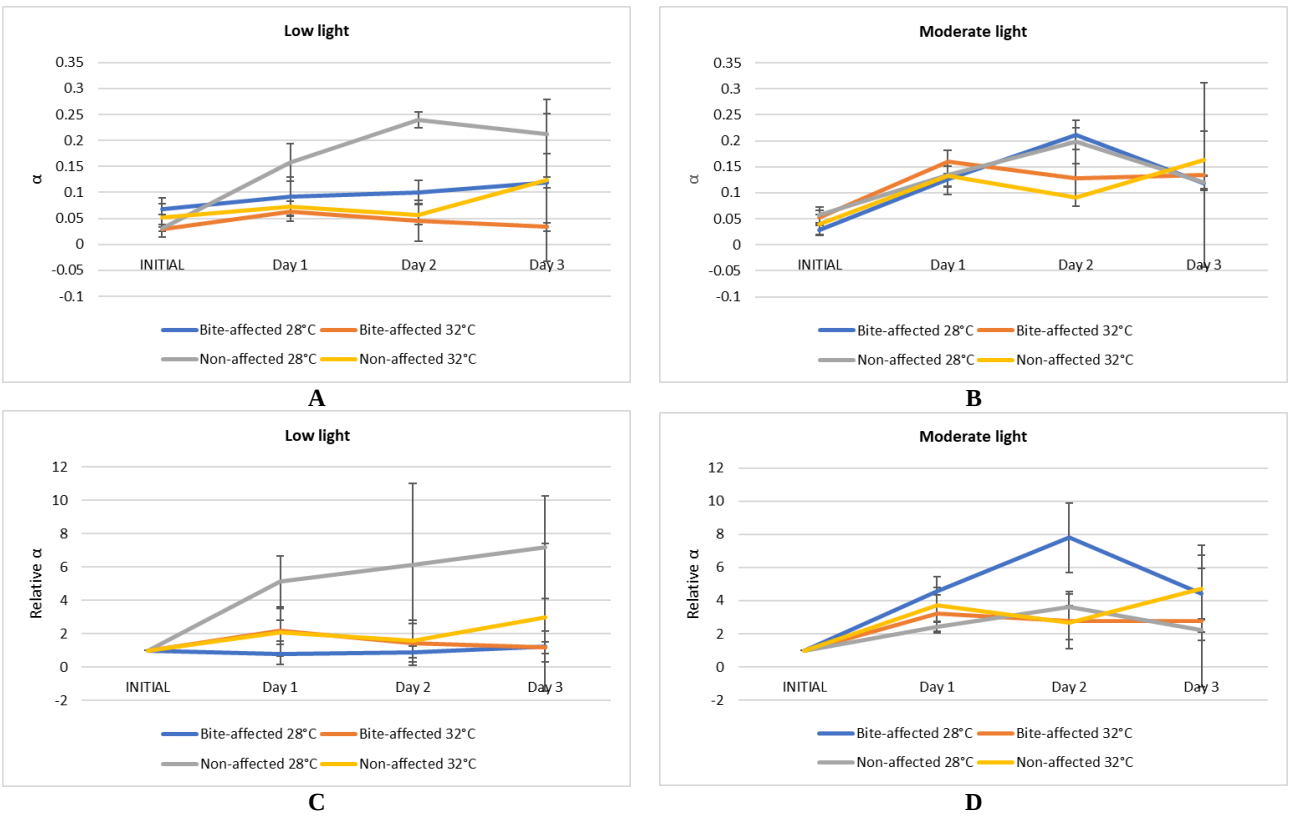

Figure 8. Alpha (α) of BA and NA parts at 28°C and 32°C at: A. Low light treatment; B. Moderate light treatment, and the relative α of the BA and NA parts at 28°C and 32°C at: C. Low light treatment; D. Moderate light treatment. Data represents mean ± SD (n=3)

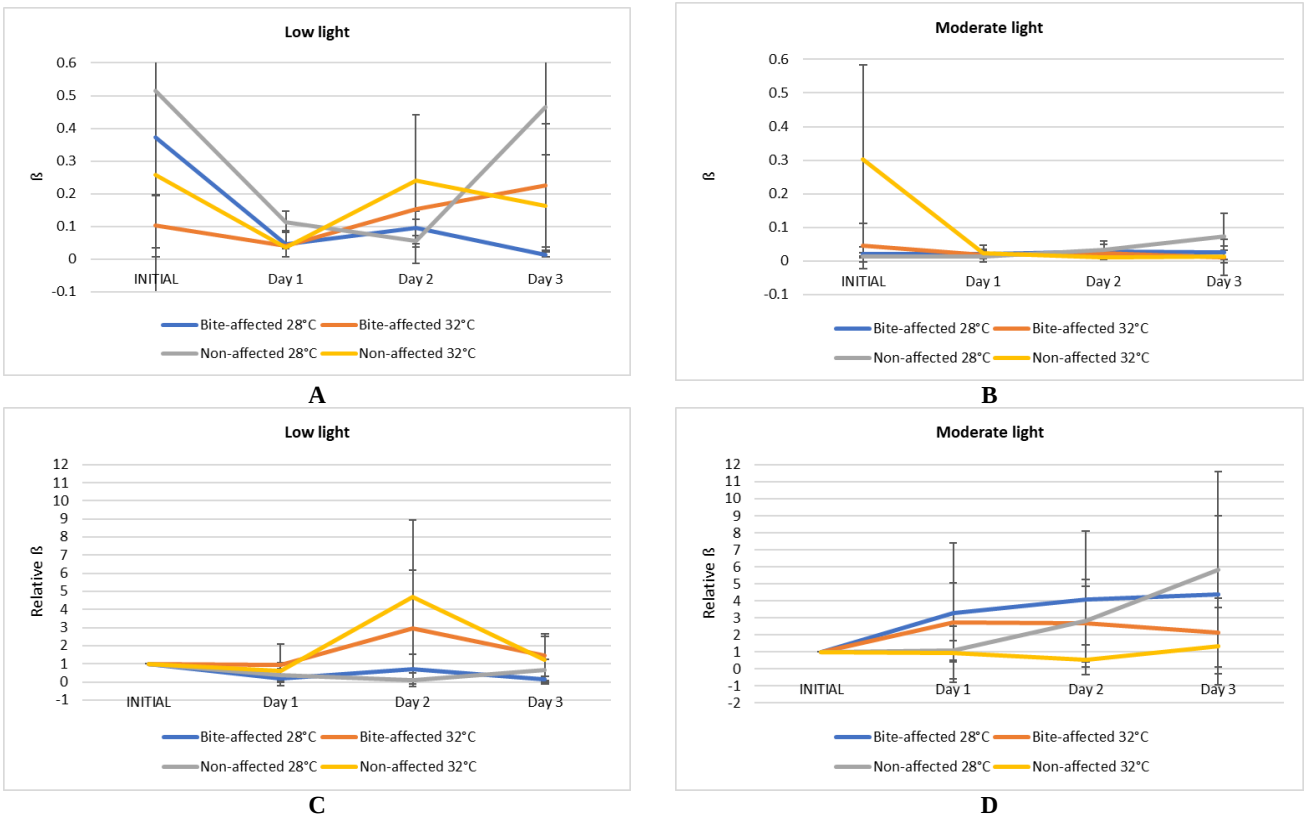

Figure 9. Beta (ß) of BA and NA parts at 28°C and 32°C at: A. Low light treatment; B. Moderate light treatment, and the relative ß of the BA and NA parts at 28°C and 32°C at: C. Low light treatment; D. Moderate light treatment. Data represents mean ± SD (n=3)

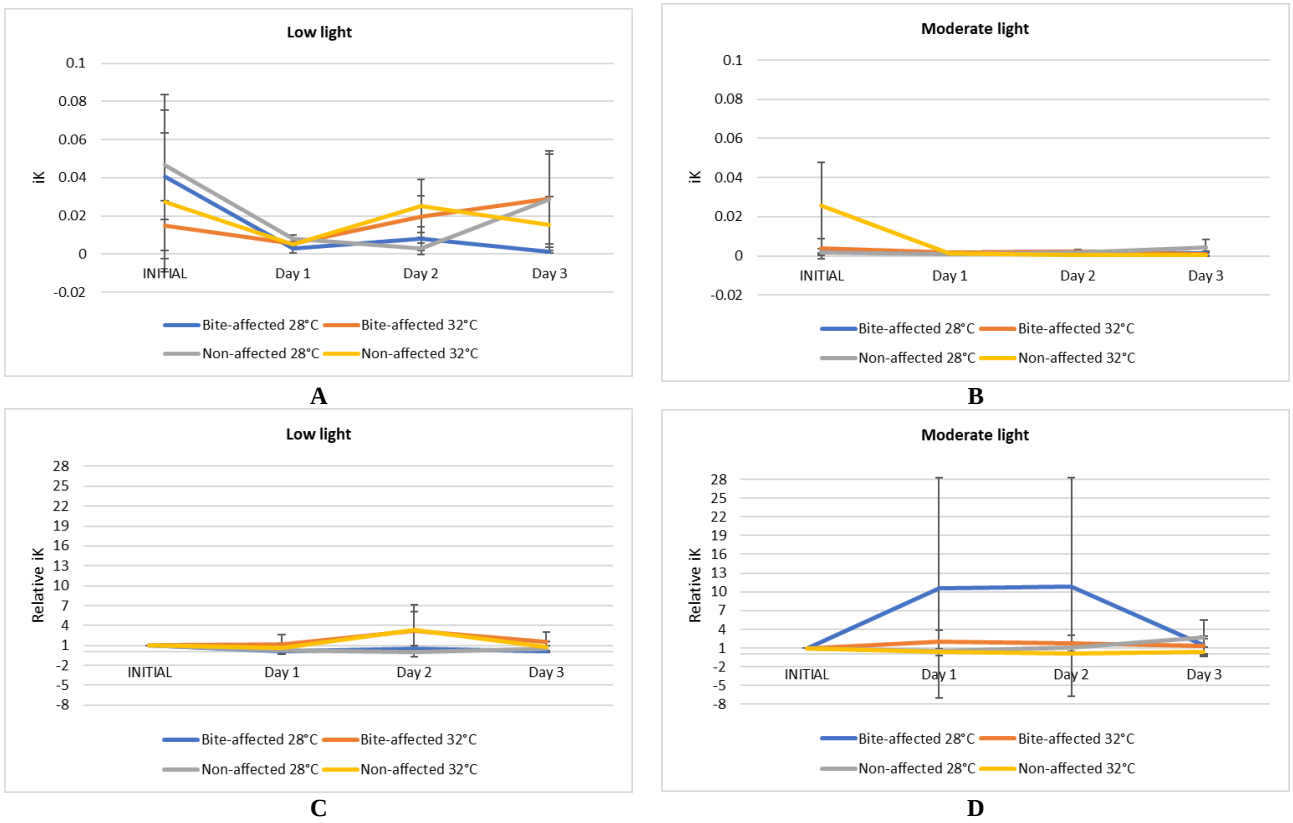

Figure 10. I_K of BA and NA parts at 28°C and 32°C at: A. Low light treatment; B. Moderate light treatment, and the relative I_k of the BA and NA parts at 28°C and 32°C at: C. Low light treatment; D. Moderate light treatment. Data represents mean ± SD (n=3)

Table 2. Three-way ANOVA for effects of different light treatments (Low light and Moderate light), temperature (28°C and 32°C) and conditions (BA and NA) on photo-physiology (F_v/F_m, $rETR_{max}$, NPQ_{max}, α, ß and I_k). P < 0.05 - *, P < 0.01 - **, P < 0.001 - ***

Dependent variables	Source of variation	*Df*	*Ms*	*F*	*P value*	
F_v/f_m	Light	1	1.01E-06	0.000	0.986	
	Temperature	1	0.011	3.416	0.068	
	conditions	1	0.037	11.793	0.001	**
	Light * Temperature	1	0.011	3.678	0.058	
	Light * conditions	1	0.009	2.765	0.100	
	Temperature * conditions	1	0.001	0.357	0.552	
	Light * Temperature * conditions	1	0.013	4.137	0.045	*
$rETR_{max}$	Light	1	2.694	4.808	0.031	*
	Temperature	1	14.596	26.053	< 0.001	***
	conditions	1	3.794	6.772	0.011	*
	Light * Temperature	1	12.439	22.203	< 0.001	***
	Light * conditions	1	0.615	1.098	0.298	
	Temperature * conditions	1	1.151	2.054	0.155	
	Light * Temperature * conditions	1	0.503	0.898	0.346	
NPQ_{max}	Light	1	0.232	6.685	0.011	*
	Temperature	1	1.763	50.834	< 0.001	***
	conditions	1	0.565	16.286	< 0.001	***
	Light* Temperature	1	0.122	3.528	0.064	
	Light * conditions	1	0.025	0.713	0.401	
	Temperature * conditions	1	0.006	0.180	0.673	
	Light* Temperature * conditions	1	0.059	1.693	0.197	
α	Light	1	0.007	1.052	0.308	
	Temperature	1	0.053	7.590	0.007	**
	conditions	1	0.001	0.202	0.654	
	Light * Temperature	1	0.030	4.291	0.041	*
	Light * conditions	1	0.003	0.402	0.528	
	Temperature * conditions	1	0.000	0.034	0.855	
	Light * Temperature * conditions	1	0.003	0.498	0.482	
ß	Light	1	0.356	11.427	0.001	**
	Temperature	1	0.000	0.007	0.936	
	conditions	1	0.060	1.930	0.168	
	Light * Temperature	1	0.072	2.312	0.132	
	Light * conditions	1	0.057	1.840	0.178	
	Temperature * conditions	1	0.017	0.549	0.461	
	Light * Temperature * conditions	1	0.021	0.663	0.418	
I_k	Light	1	0.005	18.193	< 0.001	***
	Temperature	1	5.14E-05	0.196	0.659	
	conditions	1	0.000	1.043	0.310	
	Light * Temperature	1	3.65E-05	0.139	0.710	
	Light * conditions	1	3.88E-05	0.148	0.701	
	Temperature * conditions	1	1.53E-05	0.058	0.810	
	Light * Temperature * conditions	1	0.000	0.814	0.369	

Discussion

This study tested the effects of light conditions (moderate and low light) and temperatures on the massive *P. lutea* to investigate the combined effects of ocean warming and fish predation. The results indicate no compounded effect of high temperature on predation conditions in the massive heat-tolerant coral *P. lutea.*

Following 48 hours of exposure, the BA and NA coral fragments exposed to low light and 28°C exhibited a rise in the effective quantum yield. Acclimatization most likely influenced the reactions of the massive *Porites* (Edmunds and Gates 2008). According to Denis et al. (2011), lesion healing in predation-affected *Porites* was highest during the cooling and cool periods, when radiation from the sun was lower, and sea surface temperature was moderate. This could explain how the bite-affected and non-affected coral segments responded to 28°C and low light.

The exposure to moderate light and high temperature (32°C) for 48 hours had significant effects on the BA and NA parts of the *P. lutea.* These conditions significantly affected the effective quantum yield at photosystem II, with the mean values showing a decrease. It is common to note a decrease in effective quantum yield at high temperatures (Jones et al. 1998), with the reductions induced by brief exposures to sub-lethal temperatures due to alterable photoprotective pathways (Jones et al. 1998). However, longer exposure to more severe or sub-lethal temperatures, principally at high light intensities, can cause significant biological changes and permanent decreases (Jones et al. 1998; Warner et al. 1999). This aspect may need further investigation.

Both the BA and NA parts of the *P. lutea* were affected by the exposure to moderate light and high temperature (32°C). These conditions impacted the effective quantum yield, which exhibited a decline in the mean values. Effective quantum yield reductions caused by short exposures to sub-lethal temperatures are frequently observed at high temperatures (Jones et al. 1998). Reductions in the effective quantum yield result from adjustable photoprotective mechanisms (Jones et al. 1998). However, prolonged exposure to higher or sub-lethal temperatures, particularly at high light intensities, might result in major biological alterations and long-term reductions (Jones et al. 1998). The decrease in effective quantum yield for the BA and NA components subjected to moderate light level and high temperature (32°C) indicated that these conditions did not constitute a significant threat to the photo-physiology of the *P. lutea*, and the reductions indicated alterable photoprotection. Photo-physiological processes in corals are generally affected by temperature in a curvilinear fashion which can be characterized by an abrupt positive threshold followed by a quick decay (Edmunds 2005), and alterations in temperature may produce quick responses. Jones et al. (1998) reported stress responses exhibited by corals within hours of exposure to a severe thermal episode. Additionally, when exposed to less acute chronic conditions, corals can exhibit a stress response within days or weeks (Jokiel and Coles 1990). Different coral species could show different levels of tolerance to bleaching and susceptibilities to heat stress (Marshall and Baird 2000). For instance, Hongo and Yamano (2013) demonstrated that few branching coral colonies occurring in reefs with clear water are unable to withstand severe thermal stress, while massive coral colonies generally display more tolerance, which may be explained due to the coral host having a thick tissue and/or the coral harboring a thermally robust symbiont. Additionally, Morikawa and Palumbi (2019) found that *Porites cylindrica*, a reef-building coral species had the highest tolerance compared to *Acropora hyacinthus* and *Acropora gemmifera*. Moreover, previous studies reported a decrease in the health conditions of corals when exposed to high temperatures for a longer time (Fujise et al. 2014; Zinke et al. 2015; Madeira et al. 2022), hence 48 hours of exposure to the different treatments could be insufficient to detect changes in the photo-physiology of the corals.

Predation upon corals results in damage and/or loss in the coral tissue, adversely affecting the growth and survival of coral colonies (Rotjan et al. 2006). Furthermore, physical damage caused by predation also resulted in zooxanthellae loss (Van Veghel and Bak 1993), and the reduced symbiont densities and physical damage could also interact (Rotjan et al. 2006). Moreover, when there is damage, coral colonies initiate regeneration and repair as a response mechanism, which is very energy intensive for adjacent polyps (Meesters et al. 1994). Hence these findings could explain the significant difference in Fv/Fm, rETRmax and NPQmax exhibited by the BA and NA coral pars.

The findings revealed the generalized effects of light, temperature, and predation on the photo-physiological responses of *P. lutea*; when subjected to various conditions. Similar patterns in the photo-physiology under BA and NA conditions were evident. Hence, our findings suggest that predation by fish on coral would have little negative influence on the photo-physiological susceptibility of *P. lutea* to an increase in the sea temperature. It is evident that reef fish assemblages are undergoing a rapid shift (Nystrom et al. 2000), especially when coral cover is declining worldwide and the oceans of the world are getting warmer (Hughes et al. 2003). As a result, it is essential to comprehend the synergistic effects of multiple stressors, including chronic fish grazing, rising sea surface temperature, and ocean acidification, among others, on coral survival and recovery**.**

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Prevalence and thermal photo-physiological responses of Skeletal Eroding Band (SEB)-affected *Acropora muricata* from Mauritius Island

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Abstract. *Mundil SP, Jogee SY, Kaullysing D, Bhagooli R. 2023. Prevalence and thermal photo-physiological responses of Skeletal Eroding Band (SEB)-affected* Acropora muricata *from Mauritius Island. Indo Pac J Ocean Life 7: 48-53.* The threat to coral reefs due to coral diseases has been on the rise since the late 1990s, and the effects of climate change-driven global warming and coral diseases have yet to be thoroughly investigated. This study aimed to investigate the prevalence of Skeletal Eroding Band (SEB), a coral disease resulting from the ciliate *Halofolliculina corallasia*, in *Acropora muricata*, at two sites, namely Flic en Flac and Belle Mare around Mauritius Island and the thermal photo-physiological responses, in terms of effective quantum yield at photosystem II (ΦPSII), of SEB-affected and non-affected *A. muricata*, measured with a Diving-Pulse Amplitude Modulated (D-PAM) fluorometer. Affected colonies were identified using underwater field guides, and their prevalence was estimated using a random swim method. The prevalence of SEB was highest at Belle Mare during late summer with a prevalence of 24.44 ± 1.93%. The thermal stress experiment consisting of 6 aquaria with 3 at 27°C and 3 at 32°C was set up with both SEB-affected and non-affected *A. muricata* for 19 hours. Both SEB-affected and non-affected *A. muricata* were influenced by high temperatures. However, the SEB-affected samples exhibited a higher susceptibility to 32°C treatment as the ΦPSII declined to almost zero after 6 hours of exposure. These findings suggest that the region of Belle Mare may be potentially at risk if exposed to high temperatures for extended periods and may lose up to 25% or so of its *A. muricata* cover during future thermal severe bleaching events.

Keywords: Ciliate, coral disease, Diving-PAM, ΦPSII, Mauritius, susceptibility, thermal stress

INTRODUCTION

Coral reefs are the most biologically diverse ecosystems worldwide and host over 25% of all marine organisms (Mulhall 2009), while covering <0.1% of the world`s ocean (Moberg and Folke 1999). They have high productivity and are very important as they provide various services and goods such as coastal protection with up to 97% reduction in wave energy (Ferrario et al. 2014), food, and tourist attractions (Wild et al. 2011). Coral reefs exist in various forms, and their estimated income is about USD1 billion per year (Costanza et al. 2014). Coral reefs are very important assets for Mauritius, accounting for around MUR 5 billion of the GDP (Mauritius Tourism Revenues 2020). Fringing reefs surround Mauritius with dominant species such as *Acropora muricata* (Bhagooli et al. 2019).

However, coral reefs have been in considerable decline since the 1990s and are now considered a threatened ecosystem (Coral Reefs 2015) and their loss is estimated to account for $500 billion per year by 2100 (Hoegh-Guldberg et al. 2015). In addition, they are facing several threats impacting their health and the goods and services they provide. Amongst these threats, climate change (Hoegh-Guldberg et al. 2007) is the worst, as the increasing temperatures lead to massive bleaching events that result in mass coral mortality (Hoegh-Guldberg et al. 2007). Other threats include ocean acidification (Erez et al. 2010; Veron 2011), anthropogenic activities, and a relatively novel threat, diseases (Harvell 1999; Peters 2015; Bhagooli et al. 2017). Mauritian reefs have not been spared and are expected to suffer from these threats (Bhagooli and Sheppard 2012; Bhagooli and Kaullysing 2019).

Coral disease is considered a new and emerging threat (Peters et al. 1986). The coral disease causes the deterioration of vital body functions. Ever since their first documentation, the prevalence of diseases has only been on the increase worldwide (Harvell 1999; Séré et al. 2015; Sweet and Séré 2016). Coral diseases are causing a progressive loss in coral tissue, a reduction in reproductive capacity, a decrease in the diversity of organisms associated with reefs. They also contribute to coral mortality and reduced recruitment (Harvell et al. 2007). There are now over 40 identified and described coral diseases, such as White Plague (WP), White Band (WB), Black Band (BB), Skeletal Eroding Band (SEB), and Growth Anomalies (GA), affecting over 200 reef-building species (Bruckner 2015). Studies carried out in Mauritian waters have shown the presence of GA, SEB, brown band, WP, and WB (Bhagooli et al. 2017) and white syndrome disease in *Acropora* from Saya de Malha on the Mascarene Plateau (Bhagooli et al. 2021). So far, coral diseases have only been briefly described, and most coral diseases'

etiology remains unknown (Sutherland et al. 2004; Séré et al. 2015).

SEB is a eukaryotic disease caused by a ciliate protozoan, namely, *Halofolliculina corallasia*. SEB affects the coral skeleton together with the soft tissues and is consequently named Skeletal Eroding Band. SEB was first identified in the Indo-Pacific region, including Mauritius, in 1998 (Antonius and Lipscomb 2000). A similar disease occurring in the Caribbean has also been identified. The causative agent was found to be from folliculinid ciliates (*Halafollunica* sp.), and due to its close resemblance in the way it affects the coral, it has been termed Caribbean Ciliate infection (CCI) (Cróquer et al. 2006). The typical physical trait of an infected coral is the black speckled spots that remain in the skeleton caused by their black lorica that is found in the skeleton itself (Page and Willis 2007) and algae often take over the denuded skeleton.

SEB is one of the most infectious diseases with a minimum of 82 hard coral species affected (Page and Willis 2007). Being extremely virulent and infectious, infecting nearby corals in direct and indirect contact (Antonius and Lipscomb 2000; Rodríguez et al. 2008), SEB is a disease that needs further research, especially on its effect on the health of corals and their response to external stressors such as high temperatures in the wake of a climate change-driven ocean warming phenomenon. Hence, this study aimed to assess the prevalence of and variations in photo-physiology, an established proactive indicator of coral health (Hédouin and Berteaux-Lecellier 2014), in SEB-affected and non-affected *A. muricata* to determine the effects of SEB on the health of corals when subjected to thermal stress.

MATERIALS AND METHODS

SEB Prevalence assessment

SEB field observations were carried out at three stations at each of the two study sites, namely Belle Mare and Flic en Flac, around Mauritius Island, Flic en Flac is a highly touristic site with the reef occurring some 400 m or so from the shore while Belle Mare has a long stretch of the beach and the reefs occur some 1 km from the shore. Belle Mare has some hotels and small-scale agricultural practices along its coasts, while Flic en Flac is packed with hotels and bungalows along its coast. Additionally, Belle Mare is found on the windward side and Flic en Flac on the leeward side of Mauritius (Figure 1).

Corals were identified using the "Field guides to the corals of Mauritius" (Pillay et al. 2002) and "Corals of the World" (Veron 2000). Using underwater cards for disease identification (Weil et al. 2015; Bhagooli et al. 2017) any *A. muricata* colony showing presence of SEB were identified. Since this study focused on diseases that have been studied only once, prevalence of disease was preferred to occurrence (Sutherland et al. 2004) whereby;

Prevalence = (number of diseased coral colonies÷30) x100

The survey consisted of a free swim in a randomly selected direction and observing the first 30 colonies of *A. muricata* encountered. In addition, prevalence studies were carried out in triplicate in 3 distinct regions of each site, namely near the coast, lagoon, and reef, which were selected relative to the distance between the coast and the reef crest. The same observers carried out these surveys in October and November 2019 and January and February 2020.

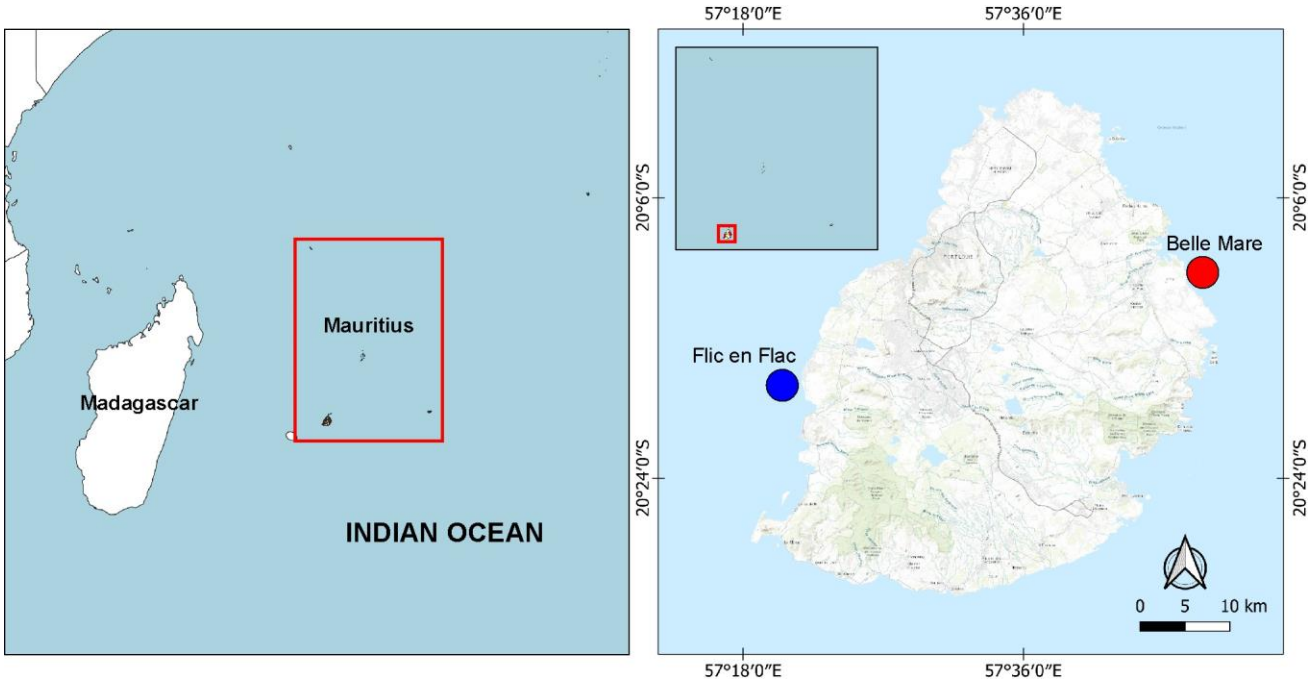

Figure 1. A map of Indian Ocean showing Mauritius (*left)*, B Map of study area (*right)*, Blue. Flic en Flac, Red. Belle Mare

Collection of samples and thermal experimental trials

In January 2020, 6 nubbins, each 3cm in length, from 3 different colonies of SEB-affected and non-affected corals were collected by snorkeling and identifying corals and then using pliers, nubbins were cut off and placed in ziplock bags. The collected nubbins were transported within 2 hours of the collection, under shaded conditions, in containers filled with seawater to laboratory facilities for thermal experimental trials.

The collected coral samples were put in aquaria fed with a constant air supply at room temperature for 3 hours to acclimatize. The coral samples were distributed in six aquaria with constant air supply and thermostats set for adjusting to the target temperatures. Coral fragments were put in the aquaria, and the thermostat was switched on to reach the desired temperatures of 27℃ and 32℃ in 3 aquaria for each temperature and, this marked the initial time prior to stress, i.e., marking T0. The corals were then continually observed for the 19 hrs duration of the experiment.

Chlorophyll *a* fluorescence measurement

A Diving Pulse-Amplitude-Modulated fluorometer (D-PAM, Waltz) was used to measure each coral fragment's effective quantum yield of photosystem II (ΦPSII). A saturation pulse (4000 µmol quanta $m^{-2}s^{-1}$) was applied by placing the probe flat to the surface of the coral sample, a few cm away from visible SEB lesions, while a probe was placed 3cm from the base for healthy corals while noting the physical appearance of the corals. The D-PAM sent a weak flash of light of <1 µmol quanta $m^{-2}s^{-1}$ at first and determined the fluorescence yield, F_t, and then determined the light adapted maximum fluorescence $F_{m'}$. The ΦPSII was then calculated as follows (Genty et al. 1989):

$$\Phi PSII = (F_{m'} - F_t)/ F_{m'}$$

Readings and observations were carried out at initial (t=0), after 3 hours (t=3), 6 hours (t=6) and 19 hours (t=19). The experiment was set up to minimize all fluctuations due to light variation by maintaining shaded conditions.

Statistical analyses

Normality of all variables was tested using Shapiro-Wilk test. The response in the ΦPSII of the SEB-affected and non-affected *A. muricata*, when exposed to 27℃ and 32℃ were compared using two-way ANOVA and Tukey HSD test. The SEB disease prevalence throughout summer was compared using Wilcoxonn-Mann-Whitney as data were non-parametric. All tests were performed in R-studio V3.6.2.

RESULTS AND DISCUSSION

Prevalence of SEB in *Acropora muricata*

SEB was observed with varying prevalence across the different stations at both studied sites. Both BM and FEF had no recorded prevalence of SEB at the reef stations. FEF had lower prevalence of SEB with a maximum prevalence of 4.44±1.92%, whereas BM had a maximum prevalence of 24.44±1.93% in February 2020 at its lagoon stations (Figure 2). An increase of 83.33% in SEB prevalence in the lagoon was observed in February 2020 when compared to October 2019 (13.33±3.33%). Overall, the lagoon stations of each studied site had the highest average SEB prevalence when compared to the near coast and reef stations.

Photo physiological response to thermal stress

Significant variations between ΦPSII of corals exposed to 27℃ and 32℃ ($p < 0.05$) were observed as from 3 hours, with corals exposed to 32℃ showing decrease in their ΦPSII. This decrease continued until the corals exposed to 32℃ were all bleached. Comparatively, the corals exposed to 27℃ had no significant variations ($p > 0.05$) in their ΦPSII (Figure 3). SEB affected and non-affected *A. muricata* all maintained a relatively similar effective quantum yield (ΦPSII) at 27ºC throughout the whole experiment. Overall, there was no significant difference ($p > 0.05$) in the ΦPSII of any coral exposed to 27℃ during the 19 hours of exposure (Figure 4).

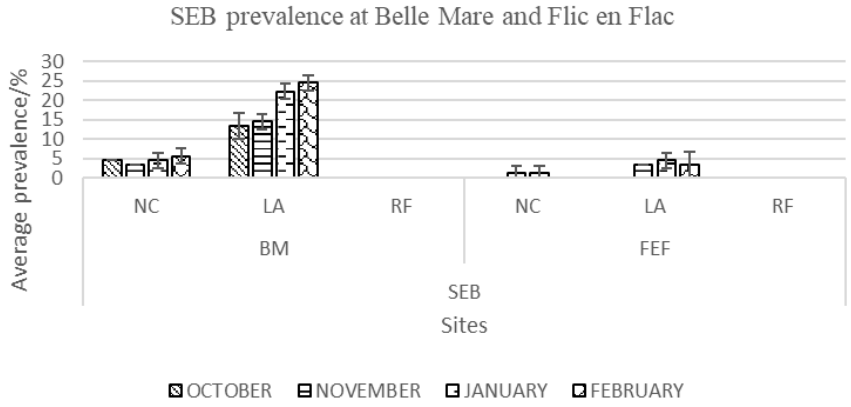

Figure 2. Mean Skeletal Eroding Band (SEB) prevalence across studied stations, Near-Coast (NC), Lagoon (LA) and Reef (RF) at the studied sites, Belle Mare (BM) and Flic en Flac (FEF). Bars represent mean±SD (n=3)

Table 1. The observed conditions of samples exposed to temperatures of 27 and 32°C at 0, 3, 6 and 19 hours

Temperature (°C)	Condition	Exposure Time (hrs)			
		0	3	6	19
27	Healthy	Normal	Normal	Normal	Normal
	SEB	Normal	Normal	Normal	Normal
32	Healthy	Normal	Paled	Bleached	Dead
	SEB	Normal	Bleached	Dead	Dead

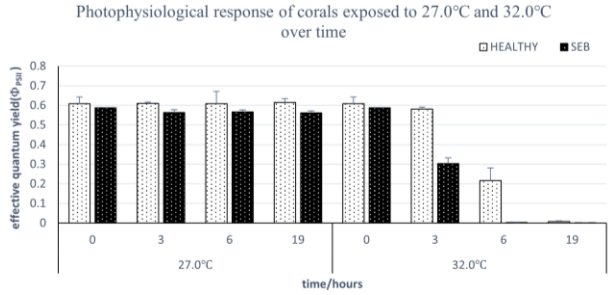

Figure 3. Effective quantum yield at PSII for healthy and SEB affected *Acropora muricata* when exposed to thermal stress. Bars represent mean±SD (n=3)

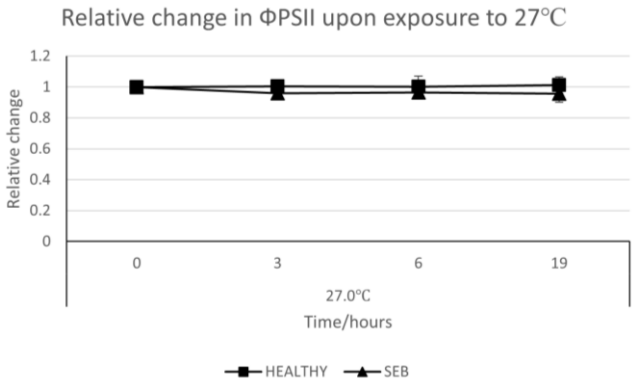

Figure 4. Relative change to initial in effective quantum yield at PSII of healthy and SEB affected *Acropora muricata* under exposure to 32°C. Bars represent mean±SD (n=3)

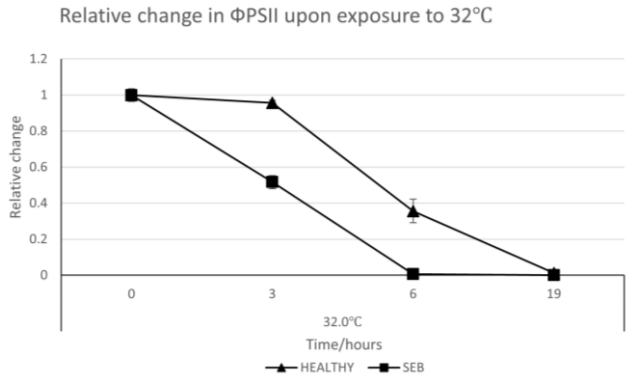

Figure 5. Relative change to initial in effective quantum yield at PSII of healthy and SEB affected *Acropora muricata* under exposure to 27°C. Bars represent mean±SD (n=3)

At 32°C, there was an overall significant decrease in the ΦPSII of both SEB-affected and non-affected corals compared to their initial, with >99% decrease after 19 hours (Figures 3 and 5). After 3 hours, a significant decrease ($p < 0.05$) in ΦPSII of the SEB-affected coral was recorded with a drop of 48.48±12.65%, while in the non-affected corals, the value dropped insignificantly ($p > 0.05$), i.e., by only 4.49±3.12%. However, the ΦPSII of SEB-affected *A. muricata* fell completely with >99% decrease after only 6 hours, while the non-affected coral decreased by 66.8%. The SEB-affected and non-affected corals following 19 hours of treatment had their ΦPSII dropping by >99% (Figures 3 and 5). An overall relative change of <0.01 was observed for both studied samples after 19 hours when exposed to 32°C (Figure 5).

Visual observations during thermal stress experiment

There was no physical change in any samples exposed to 27°C throughout the experiment, with nearly similar color even after 19 hours. SEB-affected *A. muricata* were also pale in color compared to non-affected ones. After 6 hours, partial bleaching and paling occurred in SEB-nonaffected *A. muricata*, while SEB- affected *A. muricata* was completely bleached, accompanied by complete tissue detachment from the skeleton, making the samples turn whitish and the water in the aquaria murky. After 19 hours, the SEB-nonaffected samples suffered from complete bleaching accompanied with tissue sloughing. The physical state of both samples during the experiment is summarized in Table 1.

Discussion

Previous studies found a prevalence of 8.9±1.2% of SEB at Belle Mare (Bhagooli et al. 2017) while this study found the lowest prevalence of 13.33±3.33%. This increase in SEB infection may indicate worsening conditions in the lagoon of BM. There was a nearly 2-fold significant increase ($p < 0.05$) in SEB prevalence in the lagoon of BM from early summer to mid-summer, accompanied by an increase in temperature. In this case, a rise of 3°C from October 2019 to February 2020 was noted. Furthermore, a general observation of disease outbreaks and high thermal anomalies has been noted regardless of the geographic location of these anomalies (Howells et al. 2020). This observation goes hand in hand with other observations, namely at the Great Barrier Reef, where a 2-fold increase in SEB was also observed in summer compared to winter (Harvell et al. 2007) and a similar observation of a 2-fold increase in the Arabian sea (Riegl 2002). A 10% increase

in infectivity of ciliates was recorded when the temperature was raised from 26°C to 30°C in a controlled environment (Rodriguez et al. 2009), which could explain the higher increase (83.33% in this study) in situ conditions where temperatures can rise above 30°C and is coupled with the presence of additional stressors.

Higher temperatures in mid-summer, combined with degraded water quality due to nutrient inputs, caused a decrease in defense of the corals and made them more prone to disease (Page and Willis 2007). Because of the lowered defense, an increase in microbial beta diversity, thus resulting in a reduced ability of the coral to regulate its microbiome (McDevitt-Irwin et al. 2017; Thurber et al. 2020). This high number of bacteria is a suitable food source for *H. corallasia* and may cause increased SEB prevalence (Antonius and Lipscomb 2000). Another reason for this rise in prevalence in the BM lagoon could be due to an increase in nautical activities owing to the higher number of visitors due to incoming tourists' arrival from December 2019 (Mauritius Tourism Revenues 2020). The corals sustain more damage than in early summer with increased activities such as snorkeling and glass-bottom trips (personal observations). Studies have shown that high touristic zones with nautical activities and diving can create up to a 3-fold increase in the occurrence of SEB (Lamb et al. 2014). This damage sustained to the corals directly exposes them to *H. corallasia*, and it has been shown that damaged corals are easily infected by SEB (Page and Willis 2007).

Compared to other coral samples, which only underwent paling after 6 hours of exposure to 32°C, SEB-affected *A. muricata* bleached completely after only 6 hours. Even though a clear demarcation between infected and non-infected parts exists in SEB-affected *A. muricata*, the ciliate *H. corallasia* could be stressful to the non-affected part of the coral. Some internal stress, not visible and undetected via photo-physiological responses of zooxanthellae, may be present because of *H. corallasia*. This could be because the coral may be using part of its energy to defend itself against the progression of the disease or other stressors and, in doing so, cannot have a fully functional defense against ROS/RNS, produced due to higher metabolic activities both in the host and the zooxanthellae (Traylor-Knowles and Connelly 2017). The communication between the host and zooxanthellae (Baird et al. 2009) could also be affected by the disease and hence cause a cascading effect of increased ROS/RNS production by the zooxanthellae resulting in their release because the host may be unable to cope with the oxidative stress and damage (Buddemeier and Fautin 1993).

This study has shown that SEB is a very virulent disease and in the presence of favorable conditions, there can be as much as a 2-fold increased incidence within only 4 months. Causes may be a temperature rise but also a high level of activities that have the potential to damage corals, such as snorkeling. This implies that well-frequented areas might see a consequent increase in the prevalence of diseases if some of the corals are already infected with SEB. It is noteworthy that SEB-affected corals are more susceptible than non-affected ones. As demonstrated by this study, corals affected by diseases, especially SEB, are more likely to bleach during a high-temperature event. This implies that BM is severely at risk, with 24.4% of SEB infection in its lagoon. In high temperatures, BM may lose about 24% of its lagoonal *A. muricata* in 6 hours, followed by higher numbers if the high temperatures persist for longer.

Future studies need to focus on the extent of the spread of the disease at more sites around the Island, along with other environmental factors such as microbial and nutrient levels of the seawater. The spread of SEB can be stopped with appropriate measures (Lamb et al. 2014). For example, a closure period in areas with intense underwater activities can be implemented to prevent the spread of the disease, or activities should be distributed in such a way that pressure on corals is equally distributed to minimize the impacts as much as possible (Page et al. 2009). Coral diseases are a serious issue. Coupled with the ever-increasing global temperatures, it can become one of the worst problems for coral reefs and the services they provide (Coker et al. 2013). Coral diseases are now distributed worldwide and need immediate action from marine scientists and conservationists (Morais et al. 2022). To prevent further degradation of corals due to coral diseases in the foreseeable future, further studies focusing on the nomenclature, description, characterization, and most importantly methods of transmission (Thurber et al. 2020) and combined effects with other factors such as lowered pH, nutrient input and high temperatures need to be carried out to come out with management solutions for coral diseases eventually.

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Differential responses of effective quantum yield to acute thermal stress in scleractinian corals including pre- and post-transplanted *Acropora muricata*

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Abstract. *Jeetun S, Ricot M, Taleb-Hossenkhan N, Kaullysing D, Flot J-F, Bhagooli R. 2023. Differential responses of effective quantum yield to acute thermal stress in scleractinian corals including pre- and post-transplanted* Acropora muricata. *Indo Pac J Ocean Life 7: 54-63.* Global climate change has had a serious impact on the health status of coral reefs and has led to the use of active reef restoration measures to remediate the decline in coral cover and assist in the recovery of depleted coral populations. This study aimed to assess the thermal photo-physiological responses of *Acropora muricata* pre- and post-transplantation from reef, lagoon, and nearshore stations to the experimental nearshore station and of four other non-transplanted coral species, namely, *Acropora cytherea*, *Galaxea fascicularis*, *Pocillopora damicornis* and *Lithophyllon repanda* from the reef. A visual assessment of dinoflagellate symbiont loss was conducted during summer bleaching events in 2011, 2016, and 2019 for *A. muricata* at the three stations, nearshore, lagoon, and reef, and for the other four corals at the reefs of Belle Mare (BM), Mauritius. The 2016 bleaching event appeared to be more severe for *P. damicornis* and *L. repanda*. A first experiment was carried out using pre-transplanted *A. muricata* from the reef, lagoon, and nearshore, respectively, in 2012, and a second one was conducted with post-transplanted *A. muricata* from the nearshore station in 2020, and *A. cytherea*, *G. fascicularis*, *P. damicornis* and *L. repanda* from the reef in both 2012 and 2020. The coral specimens were incubated at 28°C, 30°C, and 32°C for 3hrs. The results showed an enhanced photo-physiological thermo-tolerance through the measurement of the effective quantum yield of *A. muricata* following transplantation from the reef and lagoon to the nearshore station. Significantly different photo-physiological responses of the other four corals occurring on the reef were also reported between 2012 and 2020. These findings suggest that the nearshore transplanted *A. muricata* may have acclimatized, leading to enhanced thermo-tolerance when exposed to 30°C and *A. cytherea* among the test corals may have improved its thermo-tolerance at 30°C and 32°C possibly following several bleaching events. Further studies using longer experimental exposures and involving the symbiont species, antioxidant responses, symbiont cell density, and chlorophyll content along with coral genetics may shed light on possible mechanisms for such enhanced thermo-tolerance.

Keywords: Bleaching susceptibility, climate change, coral, Mauritius, PAM, resilience, thermal stress, transplantation

INTRODUCTION

Coral reefs are the most biodiverse ecosystems on Earth, supporting an estimated 25% of all marine life (Spalding et al. 2001). They offer a panoply of benefits to the marine environment and the world. They are not only highly significant for nature, but represent a very high value for humankind as they support millions of people whose lives depend on these natural resources for a source of food and income (Moberg and Folke 1999). Coral reefs form natural barriers protecting the coastlines from the damaging effects of wave action and tropical storms, preventing erosion. Coral reefs are responsible for the creation of marine biodiversity hotspots which are of great importance ecologically, economically and aesthetically that is energy-dependent on the coral-zooxanthellae symbiosis (Baumgarten et al. 2015). However, coral reefs are under high pressure from a combination of direct human impacts and global climate change (Wilkinson 2000). These stressors can affect the delicate balance between the reef corals and their symbiotic microalgae (zooxanthellae). This process, known as coral bleaching, is characterized by the loss of photosynthetic pigment and/or loss of zooxanthellae (Kleppel et al. 1989). The physiology of bleached corals is compromised and bleaching occurring over extended periods in time frequently results in high mortality rates in corals (Spalding and Brown 2015; Eakin et al. 2016).

Global climate change has had a serious impact on the health status of coral reefs through bleaching events which are recurrent and increasing in intensity (Hughes et al. 2018). Mild to moderate bleaching was reported in numerous lagoon and off-lagoon areas around Mauritius Island in 2016, although post-bleaching assessments revealed that most bleached corals recovered. The most impacted locations were Belle Mare, Flic en Flac, and Ile

aux Bénitiers, with more than 65% of their live corals partially bleached and the most affected coral genera were *Acropora* with more than 85% and 70% bleaching observed in *A. muricata* and *A. cytherea*, respectively (Obura et al. 2017). Coral communities were monitored over a 15-year period during thermal stress episodes and a 40% decrease in hard coral cover was observed, with the disappearance of three coral taxa facing the risk of extinction (McClanahan and Muthiga 2021). Similarly, overall live coral cover showed a noticeable decreasing trend between 2007 and 2009, while algal cover increased significantly at most sites (Bhagooli et al. 2021a; Bhagooli and Kaullysing 2019). A study done during the 2010 bleaching event revealed differential recovery of different corals under variable bleaching conditions at Belle Mare, stating that there was no significant change in the PSII functioning of the pale colonies of *Pocillopora damicornis* and *Galaxea fascicularis* post-bleaching while bleached *A. muricata* had a higher Fv/Fm and a faster recovery than *A. cytherea* (Mattan-Moorgawa et al. 2018). Differential coral bleaching and photo-physiological responses among corals are not restricted to around Mauritius only but have also been reported at the Saya de Malha, Mascarene Plateau, Indian Ocean (Bhagooli et al. 2021b).

Coral reefs around the world have been degraded to such an extent where natural recovery mechanisms and local conservation methods alone may not be sufficient for the preservation and restoration of the biodiversity (Goreau and Hilbertz 2005). Coral reef scientists and managers are resorting to active reef restoration as an important conservation tool to remediate the decline in coral cover and assist the recovery of depleted coral populations (Precht 2006; Edwards and Gomez 2007). The success of *A. muricata* transplantation on a concrete block at Belle Mare, Mauritius is fragment-size dependent, with fragments of size > 2.0 cm having a higher survival rate (Bhagooli et al. 2021c). However, the thermal responses of pre- and post-transplanted corals are yet to be investigated around Mauritius.

While many tropical corals exist near their upper thermal limit and are susceptible to slight variations in Sea Surface Temperature (SST) as small as 1-2°C over the typical summer maximum causing the coral-algal symbiosis to break down (Baker et al. 2008; Suggett and Smith 2011), some corals are able to resist and adapt to environmental stressors (Coles and Brown 2003; Douglas 2003; Venn et al. 2008). This adaption/acclimatization is due to several environmental factors such as bleaching history (Guest et al. 2012) and previous exposure to variable temperature regimes (Oliver and Palumbi 2011; Palumbi et al. 2014). For instance, *A. muricata* exposed to sea surface temperatures beyond 31°C (bleaching temperature) in nearshore sites were more resistant to bleaching than corals from lagoonal and reefal sites at Belle Mare, possibly suggesting acclimatization with potential restoration implications in an era of ongoing global climate change (Bhagooli and Taleb-Hossenkhan 2012). Though such variations in bleaching resilience can be used in creating bleaching-tolerant coral nurseries for restoration purposes, field observations of thermal resilience warrant further experimental verification. Therefore, this study aimed to assess the photo-physiological response of *A. muricata* post-transplantation from reef, lagoon and nearshore stations to the experimental nearshore station compared to four other reefal coral species under thermal stress conditions.

MATERIALS AND METHODS

Study area

The *A. muricata* fragments (n=5) of size 2 cm^2 each per station were collected from the nearshore, lagoon and reef stations and *A. cytherea*, *P. damicornis*, *Lithophyllon repanda* and *G. fascicularis* were collected at the reef station in March 2012 for the first experiment (Figure 1). In 2014, *A. muricata* from the nearshore, lagoon and reef stations were transplanted to the experimental nearshore station. Samples of transplanted *A. muricata* (n=5) from the nearshore station and *A. cytherea*, *P. damicornis*, *L. repanda* and *G. fascicularis* from the reef were collected for the second experiment in March 2020 (Table 1). The water depths varied between 0.5 to 1.5 m among these stations depending on tides. These locations were chosen because a difference in SST was observed at the nearshore, lagoon and reef stations at Belle Mare (Bhagooli and Taleb-Hossenkhan 2012). HOBO temperature data loggers were deployed to record temperature data during the first nine days in March in 2011, 2012, 2016, 2019 and 2020.

Bleaching survey

Bleaching observations (non-bleached, paling, partially bleached and bleached) of the test species were carried out during summer bleaching events in 2011, 2016 and 2019 at the three stations, namely nearshore, lagoon and reef for *A. muricata* and at the reef for *A. cytherea*, *P. damicornis*, *L. repanda* and *G. fascicularis* at Belle Mare (BM). Coral colonies were surveyed along three sets of 100 m^2 belt transect and a visual assessment of bleaching out of 30 colonies for *A. muricata* at all the three stations and 20 colonies for *A. cytherea*, *P. damicornis*, *L. repanda* and *G. fascicularis* at reef stations only in March 2011, 2016 and 2019 was conducted. The coral species were identified according to Corals of the World (Veron 2000) and the World Register of Marine Species (http://www.marinespecies.org/).

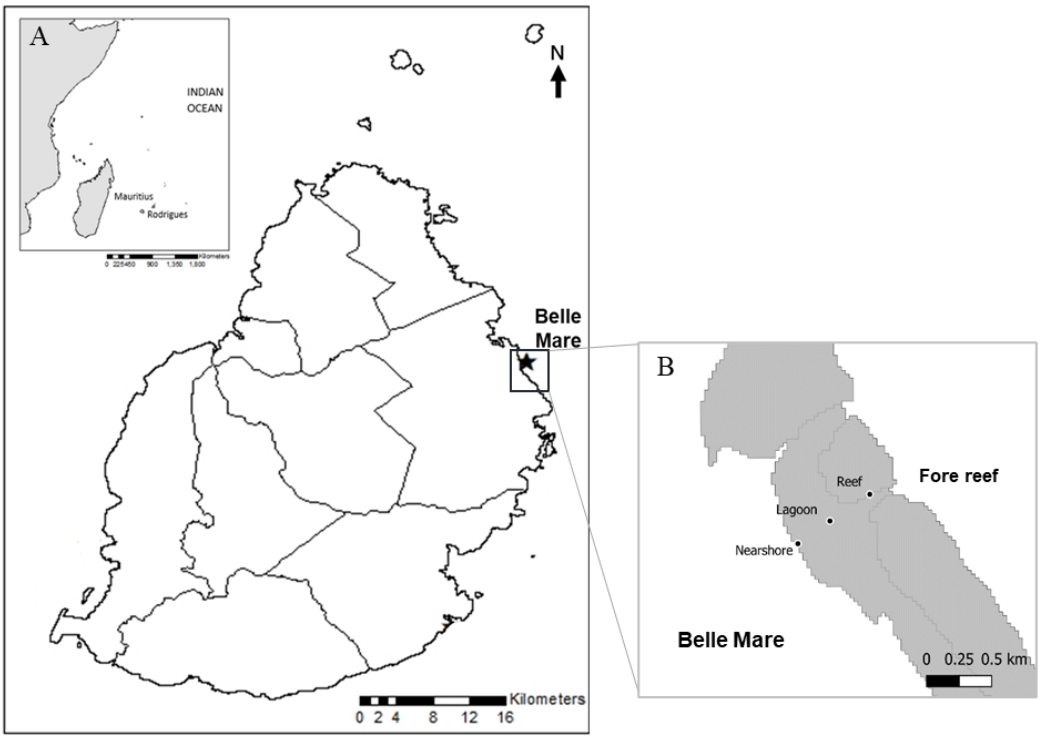


Figure 1. Location of Mauritius in the Indian Ocean (A) and the sampling sites (nearshore, lagoon and reef) (B) of transplanted *Acropora muricata* corals at Belle Mare. The grey area in (B) represents the marine waters at Belle Mare showing three stations, namely nearshore, lagoon and reef

Table 1. Transplant experimental design of *Acropora muricata* and sample collection pre- and post-transplantation for heat stress experiment. It should be noted that *Acropora cytherea*, *Pocillopora damicornis*, *Lithophyllon repanda* and *Galaxea fascicularis* were not transplanted but collected directly from the colonies

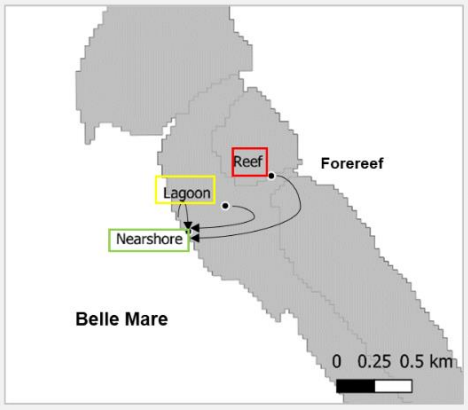
Stations	Pre-transplantation (2012)	Transplantation (2014)	Post-transplantation (2020)
Nearshore	*A. muricata* (n=5)	*A. muricata* 	*A. muricata* (n=5) Nearshore, Lagoon, Reef — Experimental nearshore
Lagoon	*A. muricata* (n=5)		-
Reef	*A. muricata* (n=5) *A. cytherea* (n=5) *G. fascicularis* (n=5) *L. repanda* (n=5) *P. damicornis* (n=5)		*A. cytherea* (n=5) *G. fascicularis* (n=5) *L. repanda* (n=5) *P. damicornis* (n=5)

Photo-physiological response

Five 2 cm^2 fragments each from parent colonies of *A. muricata* were collected from the reef, lagoon and nearshore, respectively, using pliers prior to transplantation in 2012. Coral transplantation was performed using *A. muricata* from parent colonies from the reef, lagoon and nearshore (20 m from the shore) to the experimental nearshore site at BM in 2014 (Bhagooli et al. 2021c). Transplanted *A. muricata* (n=5) were collected from the nearshore station in 2020. The *A. cytherea*, *G. fascicularis*, *P. damicornis* and *L. repanda* fragments were collected from the reef station. The coral specimens were incubated at different temperature conditions of 28, 30 and 32°C for 3hrs (Bhagooli and Hidaka 2003). The SST ranges from 28.1°C in March to greater than 30°C, the highest recorded temperature in the lagoon of BM at which bleaching onsets (Bhagooli and Taleb-Hossenkhan 2012; Bhagooli et al. 2021c). The effect of temperature on photo-physiological responses of the *in hospite* zooxanthellae was assessed using chlorophyll *a* fluorescence determined by Pulse-Amplitude-Modulated Fluorometry (Genty et al. 1989; Bhagooli et al. 2021d). The effective quantum yield of photosystem II (ΦPSII) measurements were taken initially and after 3-hr stress exposure.

Data analysis

The Shapiro-Wilk test was performed to test for normality of data. The effective quantum yield (ΦPSII) of each species relative to their initials were calculated and were arcsine (square-root) transformed before Analysis of Variance (ANOVA) to ensure normality. The Tukey *post hoc* test was performed after statistical significance to compare means between groups (IBM SPSS 21).

RESULTS AND DISCUSSION

Temperature variations at Belle Mare stations

The temperature trends at the beginning of March 2011 indicated that the nearshore and lagoon stations experienced temperatures of 32°C and 31°C, while the reef station reached at least 30°C (Figure 2A). Seawater temperatures in 2012 reached 30°C at the nearshore and lagoon stations while the reef station was about 29°C (Figure 2B). In 2016, seawater temperatures were mostly at 31°C, with nearshore and lagoon stations quite often reaching 32°C (Figure 2C). In 2019, the nearshore station tended to approach 32°C for the first three days and remained below 31°C for the rest of the days, while at the reef station it was 30°C or less (Figure 2D). In 2020, five out of nine days seawater temperatures reached 31°C or above (Figure 2E).

Bleaching survey

During the 2010-2011 bleaching event, bleaching was observed in two corals species only, *A. muricata* and *A. cytherea* (Table 2). In 2016, bleaching was observed in all selected coral species at BM. The *A. muricata* and *A. cytherea* were the most susceptible to bleaching whereas *L. repanda* was the least affected (Figure 3). A two-way ANOVA was used to test the effect of year (2011, 2016 and 2019) and species on the percentage bleaching (Table 3). There was a significant difference in the percentage coral colonies bleached between the bleaching years ($p<0.001$). Tukey *post hoc* test revealed that the percentage bleached colonies was higher (0.98 ± 0.04%) in 2016 compared to 2011 and 2019 (0.22 ± 0.04%).

Relative effective quantum yield of transplanted *A. muricata*

The effective quantum yield indicated declines at 30°C and 32°C 3-hr treatments for the pre-transplanted corals in 2012, while it is only at 32°C that such declines were observed in post-transplanted *A. muricata* samples in the 2020 experiment (Figure 4). There was a significant difference ($p < 0.05$) in relative ΦPSII in the combined effect of zones and temperature (Table 4). In 2012, the relative ΦPSII of *A. muricata* in the nearshore remained almost unchanged at 28°C and 30°C, however, decreased by half at 32°C (Figure 5A). In contrast, corals from the lagoon and reef showed a similar decrease in relative ΦPSII (0.6 times) at 30°C compared to 28°C, and a 0.2- and 0.3-fold increase, respectively, at 32°C compared to 30°C (Figures 5B, C). In 2020, the response of nearshore-to-nearshore transplanted corals showed a similar trend when exposed to different temperature regimes, nonetheless, the relative ΦPSII at 32°C was slightly lower (9% decrease) compared to 2012. Transplanted *A. muricata* from the lagoon and reef differed in effective quantum yield relative to their respective initials when exposed to 30°C compared to their prior locations (both 80% higher). A decrease in relative ΦPSII was noted in both corals transplanted from the lagoon and reef at 32°C exposure compared to 30°C, but still exhibited a 20% higher relative ΦPSII after transplantation at 32°C.

Relative effective quantum yield (ΦPSII) of other studied coral species

Temperature treatments, year and a combination of any, had significant effects on ΦPSII (relative to their initial) of the *in hospite* zooxanthellae in different species (Table 5). In 2012, *A. cytherea* showed a 0.4-fold decrease in relative ΦPSII at 32°C compared to 28°C and remained almost unchanged in *L. repanda* (Figure 6). In 2020, an increase in relative ΦPSII was noted in all four coral species at 30°C compared to 28°C, however, they behaved differently at 32°C compared to 30°C, *A. cytherea* experienced a decrease (0.3 times) while in *G. fascicularis* and *P. damicornis* increased and remained constant in *L. repanda*. A significant difference was observed in the relative ΦPSII of the coral species between 2012 and 2020 ($p < 0.05$). *A. cytherea*, *G. fascicularis* and *P. damicornis* responded differently at 32°C in 2020 compared to 2012, a higher relative ΦPSII was noted, 0.3-fold, 0.5-fold, and 0.3-fold, respectively. As for *L. repanda*, a slightly lower relative ΦPSII was recorded.

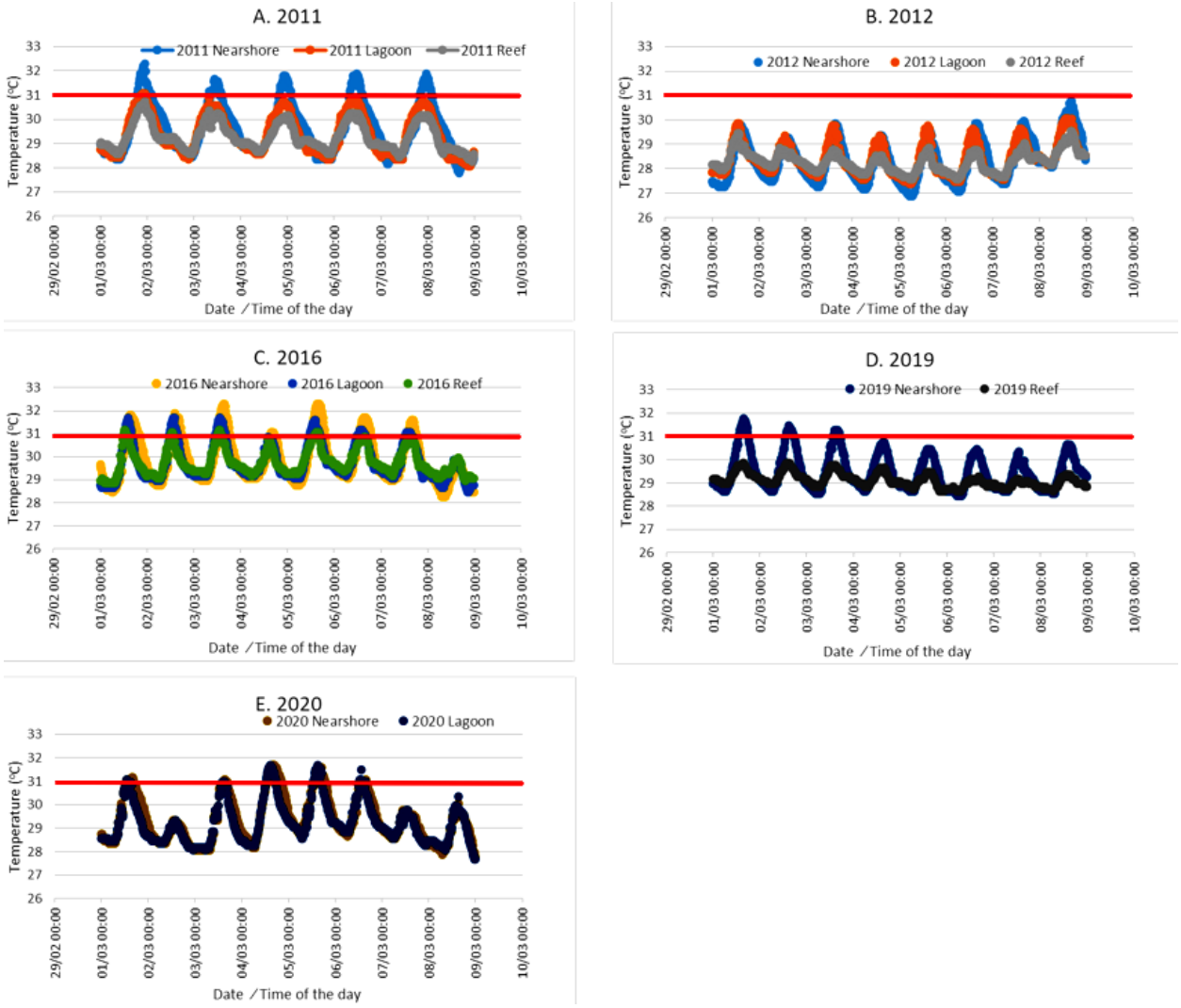


Figure 2. Temperature (°C) records at some or all the three stations (nearshore, lagoon, reef) at Belle Mare in the first week of March (01 to 09) in 2011 (A), 2012 (B), 2016 (C), 2019 (D) and 2020 (E). Red solid line represents reference line at 31°C

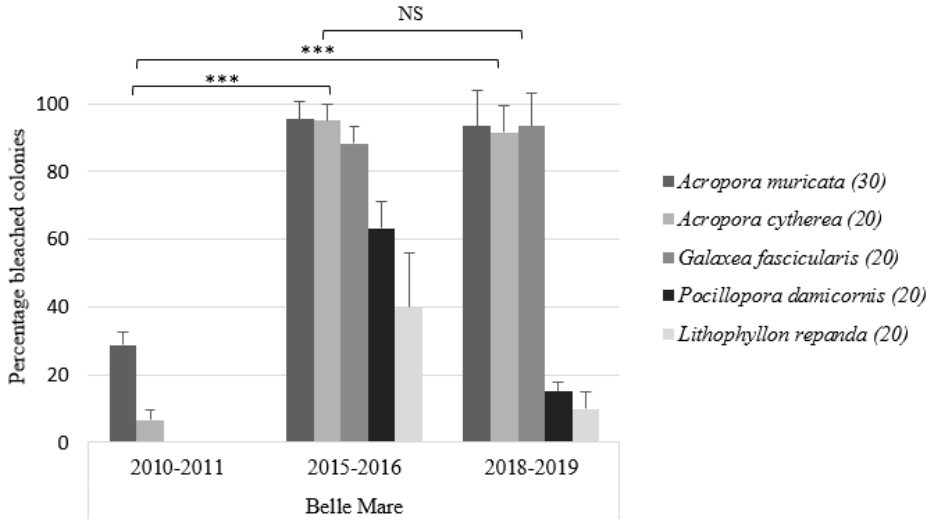


Figure 3. Percentage bleaching of five coral species (out of 30 colonies for *A. muricata* and out of 20 colonies for *A. cytherea*, *P. damicornis*, *L. repanda* and *G. fascicularis* within a 100m^2 area) at BM reefs during 2011, 2016 and 2019 bleaching events. Data represent mean ± SD (n=3)

Table 2. Bleaching observations of the coral species at BM during 2011, 2016 and 2019 bleaching events

Species	Bleaching year		
	2010-2011	2015-2016	2018-2019
A. muricata (nearshore)	Non-bleached	Non-bleached/paling	Non-bleached
A. muricata (lagoon)	Bleached	Bleached	Bleached
A. muricata (reef)	Bleached	Bleached	Bleached
A. cytherea (reef)	Non-bleached	Bleached	Bleached
P. damicornis (reef)	Paling	Bleached	Bleached
L. repanda (reef)	Non-bleached	Bleached	Bleached
G. fascicularis (reef)	Paling	Bleached	Bleached

Table 3. Two-way analysis of variance (2-way ANOVA) of effects of species and year on the percentage bleaching at BM reef

Source of variation	ANOVA			
	DF	MS	*F*	p
Species	4	1.053	103.760	<0.001***
Year	2	3.963	390.359	<0.001***
Species × Year	8	0.141	13.924	<0.001***

Note: p < 0.05 *, p <0.01 **, p < 0.001 ***, NS- not significant

Table 4. Three-way analysis of variance (3-way ANOVA) of the effects of temperature (28, 30 and 32°C), zone of collection (nearshore, lagoon, reef) and year (2012 vs 2020) on the effective quantum yield (ΦPSII) of *in hospite* symbiont relative to initial, for transplanted *A. muricata* corals after 3hrs stress exposure

Source of variation	ANOVA			
	DF	MS	*F*	p
Zones	2	0.004	2.391	0.106 NS
Temperature	2	0.015	9.587	<0.001***
Year	1	0.014	8.559	0.006**
Zones × Temperature	4	0.004	2.735	0.044*
Zones × Year	2	0.006	3.515	0.040*
Temperature × Year	2	0.016	10.022	<0.001***
Zones × Temperature × Year	4	0.001	0.685	0.607 NS

Note: p < 0.05 *, p <0.01 **, p < 0.001 ***, NS- not significant

Table 5. Three-way analysis of variance (3-way ANOVA) of the effects of coral species (*G. fascicularis*, *P. damicornis*, *A. cytherea* and *L. repanda*), temperature (28, 30 and 32°C) and year (2012 vs 2020) on the effective quantum yield (ΦPSII) relative to initial following 3hrs exposure

Source of variation	ANOVA			
	DF	MS	*F*	p
Species	3	0.037	4.708	<0.001***
Temperature	2	0.041	5.236	0.012**
Year	1	0.206	26.109	0.001***
Species × Temperature	6	0.050	6.385	0.001***
Species × Year	3	0.065	8.172	0.001***
Temperature × Year	2	0.048	6.129	0.001***
Species × Temperature × Year	6	0.012	1.538	0.188 NS

Note: p < 0.05 *, p <0.01 **, p < 0.001 ***, NS- not significant

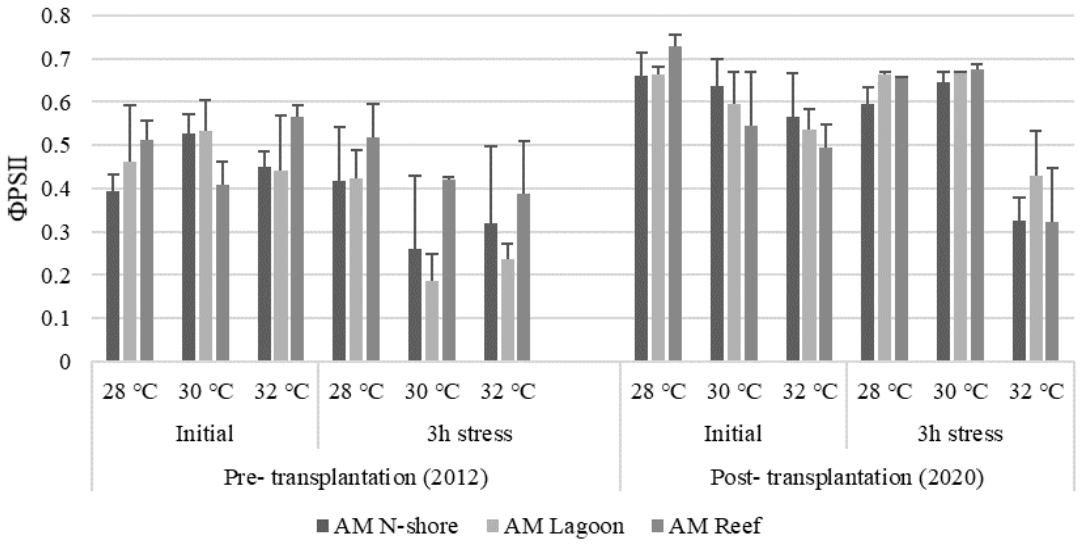


Figure 4. Effective quantum yield of pre-transplanted (2012) and post-transplanted (2020) *A. muricata* from nearshore, lagoon, reef to experimental nearshore in 2020. Data represent mean ± SD (n=3)

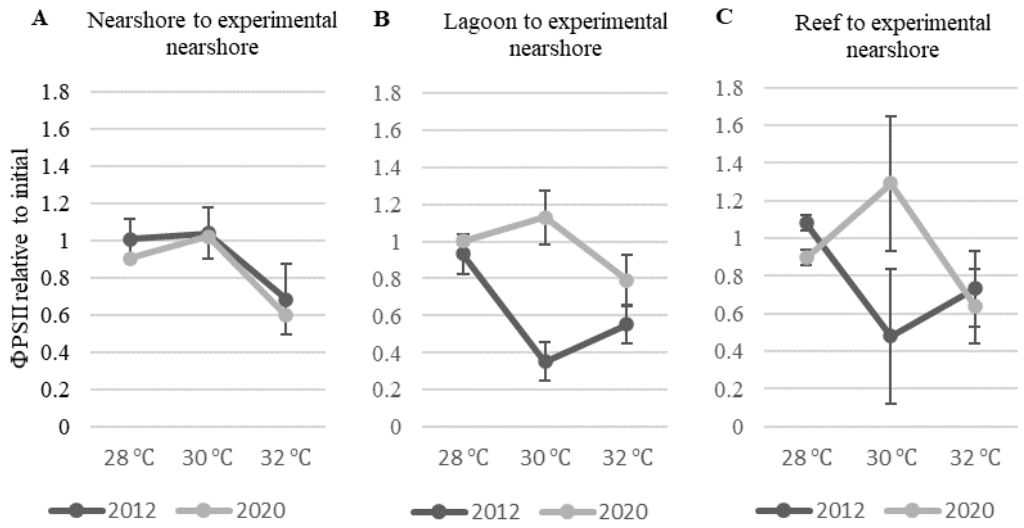


Figure 5. Relative (to initial) effective quantum yield following 3hrs exposure at 28, 30 and 32°C of pre-transplanted *A. muricata* in first experiment in 2012, and 2014 post-transplanted *A. muricata* from nearshore (A), lagoon (B), reef (C) to experimental nearshore zone in the second experiment in 2020. Data represent mean ± SD (n=3)

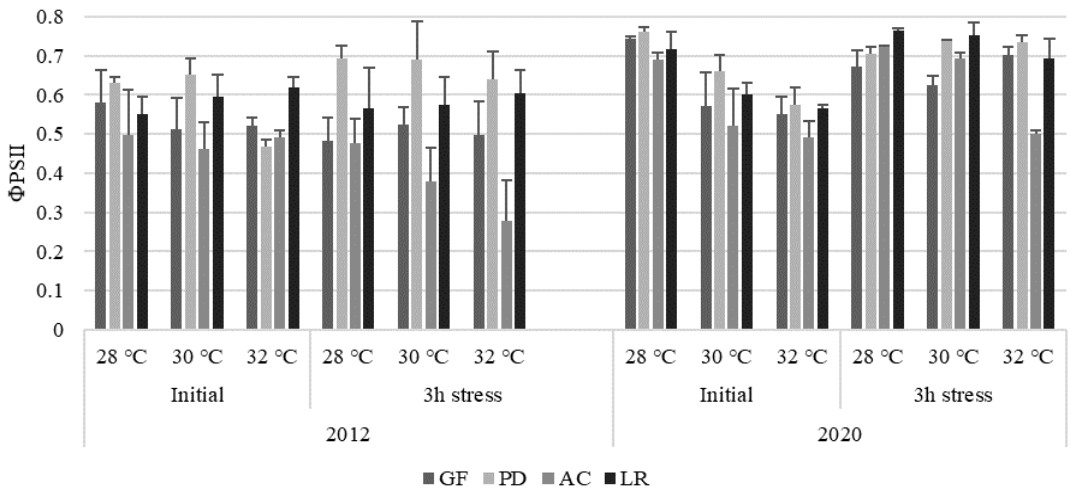


Figure 6. Effective quantum yield (ΦPSII) initially and after 3hrs exposure at 28, 30 and 32°C of *G. fascicularis*, *P. damicornis*, *A. cytherea* and *L. repanda* in years 2012 and 2020. Data represent mean ± SD (n=3)

Discussion

The results showed an increase in photo-physiological thermotolerance of *A. muricata* when the 2012 pre-transplanted stress responses were compared to the 2020 post-transplanted from reef and lagoon to the nearshore station experiments. A difference in the photo-physiological responses of other four different coral species occurring on the reef were also reported from the 2020 experiments.

In 2012, near coast samples exhibited different responses at 30°C than the lagoon and reef ones when exposed to the same temperature stress, corroborating with previous field observations by Bhagooli and Taleb-Hossenkhan (2012) at Belle Mare. It is noteworthy that Bhagooli and Taleb-Hossenkhan (2012) reported differential thermal regimes at the nearshore, lagoon and reef stations at Belle Mare. The nearshore stations experienced temperatures of above 31°C as from the second part of January 2011, while for lagoon and reef stations such temperatures were observed at the end of February 2011. At the end of February 2011, only the nearshore stations had maximal temperature of 32°C. This study reported the thermal bleaching tolerance of *A. muricata* in coastal waters compared to the lagoon and reef even though temperature records were beyond the bleaching threshold. A plausible explanation to this disparity in bleaching susceptibility may be due to acclimatization of the corals to fluctuating temperature regimes.

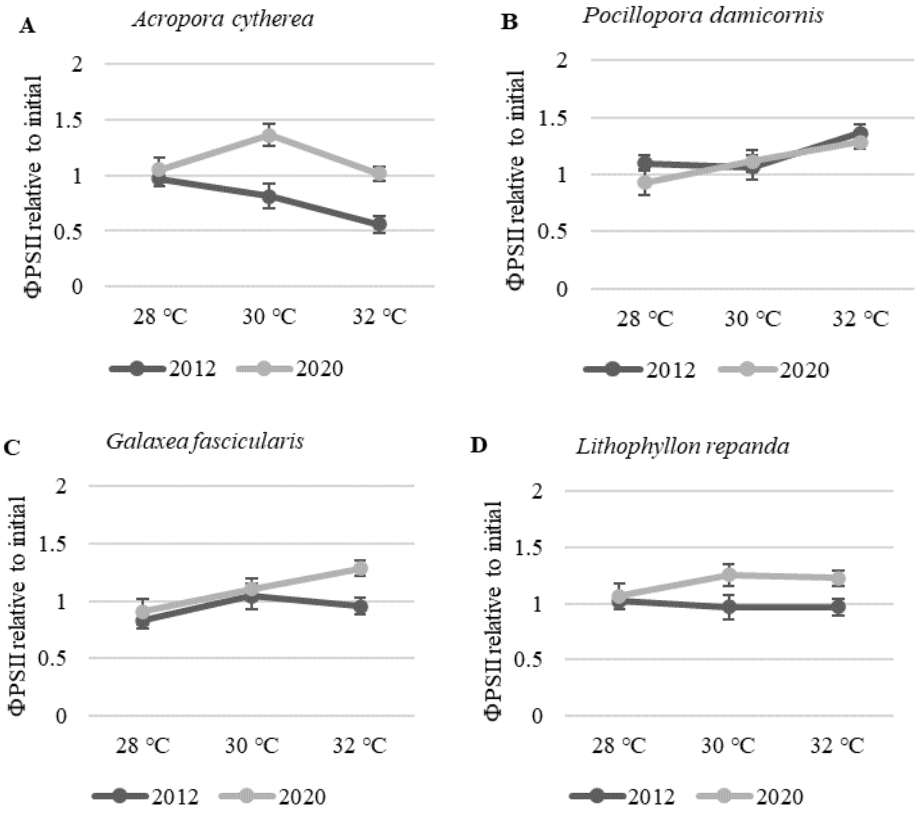


Figure 7. Effective quantum yield (ΦPSII) relative to initial of *A. cytherea* (A), *P. damicornis* (B); *G. fascicularis* (C); and *L. repanda* (E) in 2012 and 2020. Data represent mean ± SD (n=3).

It has been shown that corals subjected to a thermally dynamic environment become more resilient to thermal stress (Schoepf et al. 2015; Safaie et al. 2018). Further evidence of higher chlorophyll a content, estimated productivity, Hsp70 gene expression and protein synthesis in *A. muricata* coast colonies supports this difference in photo-physiological response (Louis et al. 2016a, b, 2020). A study documented highly variable irradiance levels at Belle Mare where lower irradiance was recorded in the coast compared to the reef flat and lagoon possibly due to variations turbidity levels (Bhagooli and Taleb-Hossenkhan 2012). These results are important as they show that thermal history, in addition to light history, can influence the response of reef-building corals to thermal stress and therefore have implications for the modeling of bleaching events.

The thermal history of the coral in a particular environment also has an influence on the response of coral symbiosis to thermal stress. Experimental studies report that pre-stressed corals had more effective photo-protective mechanisms which was associated with decreased loss of symbionts and a lower decline in photosynthetic efficiency (Middlebrook et al. 2008). Similarly, the effective quantum yield of *A. muricata* (relative to its initial) was comparatively higher after transplantation from the lagoon and reef compared to 2012. These corals have witnessed two major thermal anomalies in 2016 and 2019 since their transplantation in 2014, the 2016 bleaching anomaly being the most severe during which Belle Mare was one of the most affected sites (>65 % partial bleaching) with more than 85 % and 70 % bleaching observed in *A. muricata* and *A. cyhterea*, respectively, possibly implying the acclimatization of nearshore *A. muricata*.

The difference in photo-physiological response is at least partially dependent on the residing host symbiont (Bhagooli and Hidaka 2003; Bhagooli 2009, 2010). Zooxanthellae "clades" have been recently redefined into eleven genera (LaJeunesse et al. 2009). *Symbiodinium* (clade A) enhances the survival of the coral host to thermal stress conditions and confers them with thermal resistance (Reynolds et al. 2008). Another study in Mexico looked at *Orbicella faveolata* colonies before, during, and after a thermal anomaly and found that colonies with *Symbiodinium* A3 (*S. fitti*) as their dominant clade were less susceptible to bleaching than colonies with *Brevolium* B17 or *Cladocopium* C7 as a result of their higher maintenance in quantum yield of photosystem II (Kemp et al. 2014). In Belize, 72% of Millepores were dominant in *Symbiodinium* at sites experiencing warmer temperature (Schwiesow et al. 2021). These observations are in line with previous reports of *Symbiodinium* sp. at both coast and reef of Belle Mare (Louis et al. 2016a,b). However, further genetic and microbiome analyses are warranted to

characterize the algal symbiont residing in the coral species from Belle Mare.

Variable bleaching susceptibility pattern were observed in different taxa (Loya et al. 2001; Bhagooli and Yakovleva 2004; Yakovleva et al. 2005; Pratchett et al. 2013). The order of bleaching susceptibility based on their photo-physiological response to thermal stress were as follows: *A. cytherea* > *G. fascicularis* > *P. damicornis* > *L. repanda*. Similar results were highlighted in a study during the bleaching anomaly in 2009 at Belle Mare reporting *A. cytherea* as most susceptible and *P. damicornis*, *G. fascicularis* and *L. repanda* as more tolerant to thermal stress and mortality (Mattan-Moorgawa et al. 2012). It has been suggested that *Acropora* corals with a higher skeletal surface area to volume ratio are least tolerant to high temperature anomalies compared to those with lower ratio (Fujioka 1999). Thick-tissued massive and encrusting corals have a more effective photo-protective capacity as they retract their tissue and self- shade better than thin-tissued corals (Brown 1997; Hoegh-Gulberg 1999). A study on the differential bleaching observations and post-bleaching PSII functioning recovery showed paled *P. damicornis* and *G. fascicularis*, displayed no significant changes in fluorescence quantum yield while bleached *A. muricata* recovered faster than *A. cytherea* post- bleaching (Mattan-Moorgawa et al. 2018). Bleaching susceptibility was significantly different among corals ascribed to each growth form, whereby a much higher proportion of branching, tabular and submassive corals bleached, compared to encrusting, massive and free-living corals.

It has been documented that coral hosts have the ability to shuffle (alter the relative abundance of the extant symbionts) or shift (populated by new symbionts from the environment) their endosymbionts to a more thermotolerant one, thereby becoming more resistant to thermal stress (Jones 2008; LaJeunesse et al. 2009; Cunning et al. 2015; Silverstein et al. 2015; Swain et al. 2018). A difference in response in *G. fascicularis* was noted at 32°C in 2012 in comparison to 2020, most probably due to a change in symbiont type to adapt to warming temperatures. A previous study showed a high diversity of symbiont types in *G. fascicularis* with a higher abundance of thermally tolerant *Durusdinium* symbiont prevalent at sites experiencing maximum yearly average in sea surface temperature at Hainan Island, providing evidence of symbiont shuffling to cope with changes in the environment (Zhou et al. 2017).

An increased thermotolerance of *A. muricata* after being transplanted from reef and lagoon to the nearshore station was observed. Also, a difference in the photo-physiological response of different taxa occurring on the reef was also reported from 2012 to 2020 which suggests the possible acclimatization of these coral species as a consequence of their exposure to repeated thermal anomalies and a variable temperature regime at different stations at Belle Mare. However, to further elucidate the response of these corals to thermal stress conditions additional chlorophyll *a* fluorescence parameters such as the maximum relative electron transport rate ($rETR_{max}$) and the maximum non-photochemical quenching (NPQ_{max}) could be used as done by Bhagooli and Hikada (2006). Other management factors also need to be locally under control or within appropriate limits for survival of corals, such as water quality and predators, for instance, the corallivore *Drupella cornus*, following overgrowth of macroalgae (Kaullysing et al. 2016). There are other coral eating snail species (Kaullysing et al. 2019) that may require further investigations with respect to their links with water quality, macroalgal overgrowth on corals and predation.

The findings of this study are indicative that the nearshore transplanted *A. muricata* may have acclimatized yielding enhanced thermo-tolerance when exposed to 30°C and the non-transplanted *A. cytherea* among the other test corals may have improved its thermo-tolerance at 30°C and 32°C possibly following several bleaching events. Further studies involving the symbiont species, symbiont cell density and chlorophyll content along with coral genetics may shed light on possible mechanisms for such enhanced thermo-tolerance. The mechanisms involving antioxidant/ oxidative stress consisting of enzymatic and non-enzymatic processes can also be studied to investigate how corals counteract the damaging effects of Reactive Oxygen Species (ROS) as studies have reported higher total phenolic contents and ferrous-reducing antioxidant potential in *A. muricata* from nearshore than on the reef during summer at Belle Mare (Louis et al. 2016a,b). In addition, further work to mimic diurnal variation in temperature and light over a longer period of time is also warranted to assess the long-term response to continuous exposure to high-temperature anomalies.

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Differential photo-physiological responses of two giant clam species to elevated temperature stress from Rodrigues Island, Western Indian Ocean

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Abstract. *Ramah S, Kaullysing D, Soondur M, Taleb-Hossenkhan N, Bhagooli R. 2023. Differential photo-physiological responses of two giant clam species to elevated temperature stress from Rodrigues Island, Western Indian Ocean. Indo Pac J Ocean Life 7: 64-70.* Bleaching events leading to mass mortality of coral reef and its associated symbiotic organisms have become an alarming issue worldwide. However, as compared to corals, little has been documented regarding giant clams' (Tridacnines) thermal photo-physiological susceptibility. Triplicate specimens of the small giant clam *Tridacna maxima* and the fluted giant clam *T. squamosa* collected from the lagoon of Rodrigues Island were exposed at two temperatures, 29°C and 32°C, under a constant low light intensity of approximately 200 µmol quanta m^{-2} s^{-1} over a 12-hour duration. The photo-physiological parameters, namely, effective quantum yield of photosystem II (Φ_{PSII}), relative maximum electron transport rate ($rETR_{max}$) and maximum non-photochemical quenching (NPQ_{max}) were determined using a diving Pulse-Amplitude-Modulated (D-PAM) fluorometer prior to and after 3 and 12 hours of exposure. At 29°C, the photo-physiological parameters did not vary significantly for both species. At 32°C, *T. squamosa* and *T. maxima* exhibited significant declines in Φ_{PSII} at 3 and 12 hours, respectively. The $rETR_{max}$ of *T. squamosa* showed a significant decrease at 3 hours, while both species showed a significant reduction in their NPQ_{max} functioning after 3 hours. The experiment also recorded the disintegration of the mantle tissue in *T. squamosa* after 12 hours. These findings indicate that *T. squamosa* is thermally more susceptible than *T. maxima*. Further in-depth investigations on symbiont genetic types and antioxidant responses of both the *Tridacna* host and symbionts are required to thoroughly understand giant clams' variable heat stress responses in an era of ocean warming.

Keywords: D-PAM, Tridacnines, tropical island, thermal stress, photo-physiology

INTRODUCTION

Coral bleaching, a general environmental stress response through the loss of symbionts and/or their photosynthetic pigments, has become an alarming issue resulting in mass coral mortality worldwide and not sparing Mauritian waters (Bhagooli and Sheppard 2012; Bhagooli and Kaullysing 2019; Bhagooli et al. 2021a,b,c). Many environmental factors such as elevated seawater temperatures (Gates et al. 1992; Brown et al. 1995, Bhagooli and Hidaka 2003, 2004; Hoegh-Guldberg and Bruno 2010), high light intensity (Hoegh-Guldberg and Smith 1989; Brown et al. 2000; Bhagooli and Hidaka 2004), salinity stress (True 2012; Gegner et al. 2017), cold shock (Gates et al. 1992; Kobluk and Lysenka 1994), and disease (Bhagooli et al. 2021d; Neely et al. 2021) are known to be responsible for coral bleaching. However, high sea surface temperature is believed to be the leading factor for coral bleaching. Many researchers have attempted to clarify the mechanism of coral bleaching, especially under conditions of elevated seawater temperature (e.g., Lesser 1997; Ralph et al. 2001; Bhagooli and Hidaka 2004; Downs et al. 2009; Bhagooli 2013). Experiments have demonstrated that elevated seawater temperature is a primary trigger of coral bleaching. However, many of these thermal stress experiments were performed at seawater temperatures greater than 32°C. Under such harsh thermal stress, a large number of Symbiodiniaceae were most likely expelled due to host cell detachment, and the subsequent loss of Symbiodiniaceae from coral tissues led to coral bleaching (Gates et al. 1992; Bhagooli and Hidaka 2004; Fujise 2013). Many natural coral bleaching events can occur even under conditions of moderate thermal stress, 1-2°C higher than the average ambient seawater temperature, for prolonged periods of time (Goreau and Hayes 1994; Winter et al. 1998; Lough 2000; Ward et al. 2002).

Climate change represents a challenge that may negatively affect the recovery of giant clams. It has been reported that the collective impact of ocean acidification and high seawater temperatures reduces calcification (Rodolfo-Metalpa et al. 2011; Mackenzie et al. 2015), fertilisation and development (Kurihara et al. 2007; Parker et al. 2009), and growth and metabolism (Talmage and Gobler 2011; Clark et al. 2013) in marine bivalve molluscs. Being a reef dwelling organism, giant clams will most likely face the impacts of climate change (Hoegh-Guldberg et al. 2007) causing a decline in their occurrence on reefs

(Ramah et al. 2019). Even though most studies have concentrated on temperature effects on corals, a recent focus has been the responses to heat stress in giant clams (Eckman et al. 2014; Pappas et al. 2017; Andrefouet et al. 2017). Unlike corals, giant clams host the symbiotic Symbiodiniaceae extra-cellularly, within zooxanthellal tubes in the mantle and stomach (Norton et al. 1992). Nonetheless, similar to corals, giant clams have also been affected by global ocean warming (Watson and Neo 2021) since they depend on symbiotic Symbiodiniaceae (Blidberg et al. 2000). Exposure of giant clams to elevated temperatures has led to their mass mortality around the world (Junchompoo et al. 2013).

The coastal waters of Rodrigues Island have not been spared by the ocean warming phenomenon. The effects of climate change on giant clams are still to be thoroughly understood, especially with respect to their photo-physiological responses (e.g. Bhagooli et al. 2021c,d) and their survival. This study therefore investigated the thermal susceptibility of the *in-hospite* Symbiodiniaceae of two giant clam species, *Tridacna maxima* and *T. squamosa*, from Rodrigues Island in a view to understand giant clams' survival and the process underpinning their declines worldwide.

MATERIALS AND METHODS

Study site and samples collection

Six specimens from each of the two species of giant clams, *Tridacna maxima* and *T. squamosa*, were morphologically identified *in situ* according to Ramah et al. (2017) and collected following a modified protocol of Trench et al. (1981) from the lagoon of the South-East Marine Protected Area (SEMPA) in Rodrigues (Figure 1). The sites were selected based on the availability of the two targeted giant clam species. The shell of both species collected ranged from 9.3 ± 0.23 cm to 8.2 ± 0.42 cm in length for *T maxima* and *T. squamosa*, respectively. Care was taken to minimize damage and trauma to the organisms when they were being removed from their substrate. The samples were allowed to acclimatize in a seawater tank at room temperature maintained with an air pump filter for 24 hours prior to experimental trials. The responsiveness of all specimens was checked, and their initial photo-physiological parameters were measured to assess their good health before placing them in the experimental tanks.

Experimental design

Two seawater tanks were set up and maintained at an approximate temperature of 29ºC and 32ºC using heaters. The mean seawater temperature in both tanks were at 29.3 ± 0.18ºC and 31.9 ± 0.09ºC, respectively. Care was taken to keep a controlled light intensity of ~200 µmol photons m^{-2} s^{-1} using artificial light. Three specimens from the two giant clam species were placed in each experimental tank and were exposed for 12 hours (Figure 2). After 3 and 12 hours of treatment, the chlorophyll *a* fluorescence measurement for each sample was recorded.

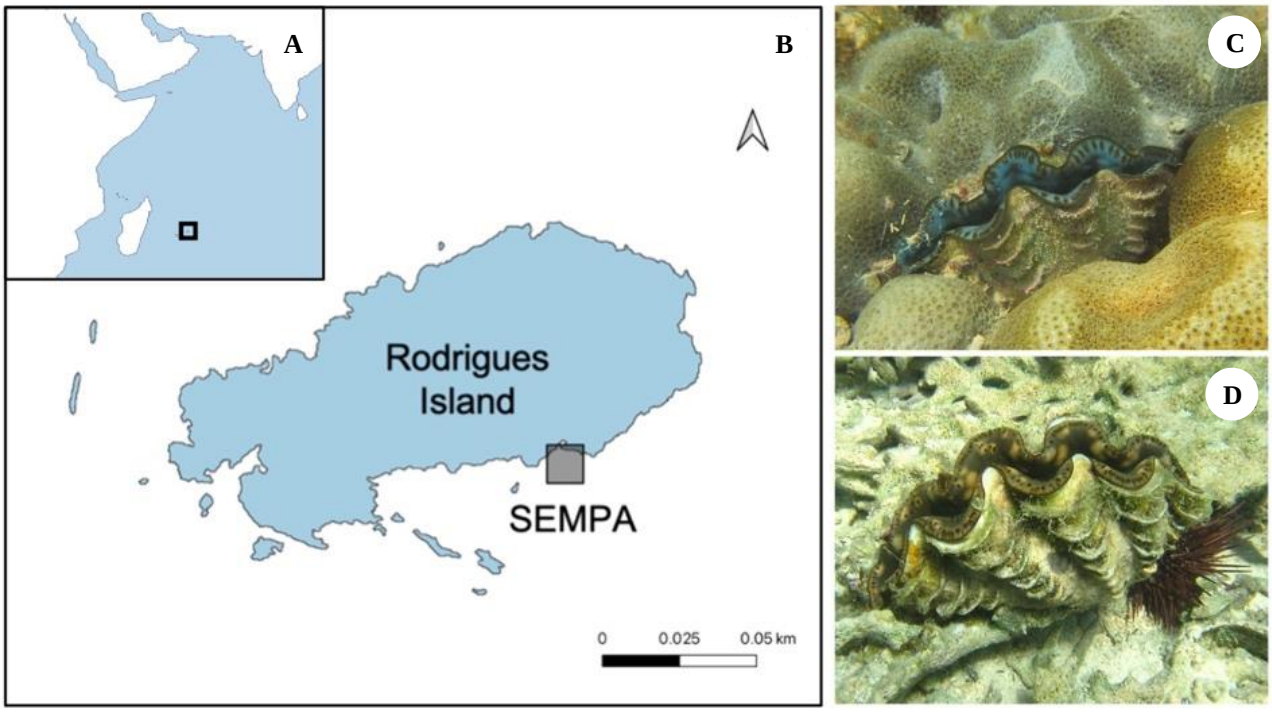

Figure 1. A. Western Indian Ocean Map showing location of Rodrigues Island (19.7245° S, 63.4272° E), B. South-East Marine Protected Area (SEMPA) location in Rodrigues Island, C. *Tridacna maxima* specimen collected for the experiment and D. *T. squamosa* specimens collected

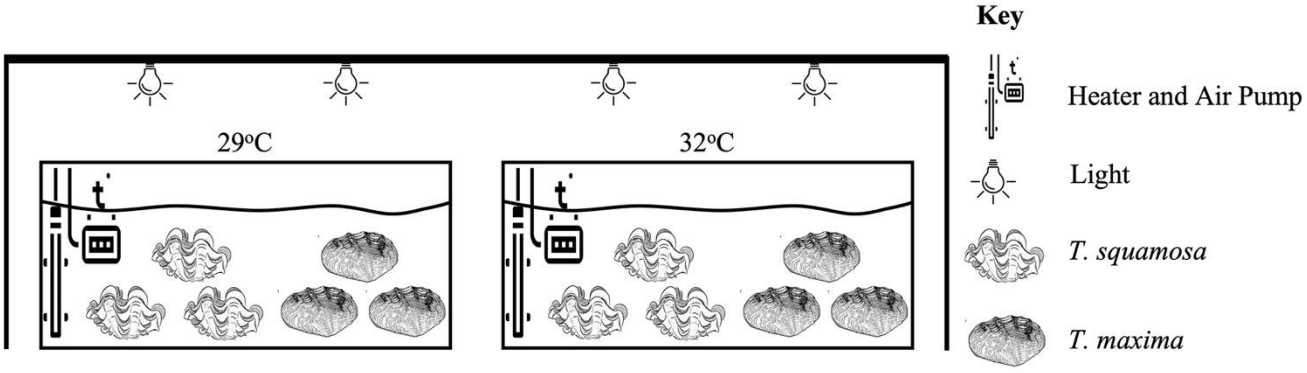

Figure 2. Experimental design for thermal experimentation of *T. maxima* and *T. squamosa* at 29°C and 32°C

Chlorophyll *a* fluorescence measurement

Chlorophyll *a* fluorescence was measured using a Diving Pulse-Amplitude-Modulated (DIVING-PAM or D-PAM) fluorometer (Walz, Germany) as an indicator of the photo-physiological responses of the giant clams to thermal stress. To ensure consistency of measurements, readings were taken within the mantle of the giant clams only where the Symbiodiniaceae are hosted. The optical fibre of the D-PAM was placed at a distance of about 1 mm (probe to mantle). F_o was measured by applying a weak pulsed red light (LED 650 nm, 0.6 kHz, 3 µs) followed by a saturating pulse of bright actinic light (4000 µmol photons m^{-2} s^{-1}, width 800 ms) which was then applied to give the maximal fluorescence value (F_m). Three main chlorophyll *a* fluorescence parameters, namely, the effective quantum yield of Photosystem II (Φ_{PSII}), the relative maximum electron transport rate ($rETR_{max}$) and the maximum Non-Photochemical Quenching (NPQ_{max}) were chosen as they are known to be widely used in photo-physiological stress studies in marine organisms (Bhagooli et al. 2021c). Φ_{PSII} is an indication of the photosynthetic efficiency of the PSII when all reaction centres are open. $rETR_{max}$ is used to measure the rate of electron transport through the reaction centres in the PSII, and NPQ_{max} gives an indication of the ability of a photosynthetic organism to dissipate excess radiation through non-damaging heat emissions (Bhagooli et al. 2021c). The photo-physiological parameters were determined initially, i.e., prior to start of the experiment and after 3 and 12 hours of exposure.

Data analysis

Data were transformed, if necessary, using arcsine-square root transformation to meet the assumptions of normality and equal variance for use of parametric statistical tests. The significant differences in the photo-physiology response (Φ_{PSII}, $rETR_{max}$ and NPQ_{max}) of the two species of giant clams at 29°C and 32°C were analyzed using a two-way ANOVA. A Tukey's comparison of means was also run using the SPSS Software (Version 6.0). P-values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Light and temperature variations

The seawater temperature and light intensity were monitored using a HOBO Pendant temperature and light data logger. The mean seawater temperature was 29.2°C ± 0.18 for the stress tank of 29°C and 31.9°C ± 0.07 for the stress tank set up at 32°C. The light intensity was maintained at a mean intensity of 197.9 ± 0.16 µmol photons m^{-2} s^{-1} for both set-ups.

Photo-physiological responses of giant clams

The chlorophyll *a* fluorescence parameter did not vary significantly (p=0.086) during the exposure duration for both test species at 29°C (Figures 3.A, C, E). However, at 32°C, differential responses between the two studied species, both at the photo-physiological and survivorship levels, were notes. *T. squamosa* showed a significant drastic decline in the photo-physiological parameters after 3 hours of exposure and exhibited signs of disintegration, indicative of mortality after 12 hours of exposure. *T. maxima* started to show a significant drastic decline in its photo-physiological parameters only after 12 hours of exposure (Figures 3.B, D, F). The results demonstrated that Φ_{PSII}, $rETR_{max}$ and NPQ_{max} were highly influenced by the species of giant clam (p<0.01) (Table 1) at 32°C. That is, both *T. maxima* and *T. squamosa* showed a significant response in their photo-physiological response at exposed temperature of 32°C. A strong significant correlation (r=0.627, p=0.013) was also found between the photo-physiology responses and the temperature exposures at 32°C for both species.

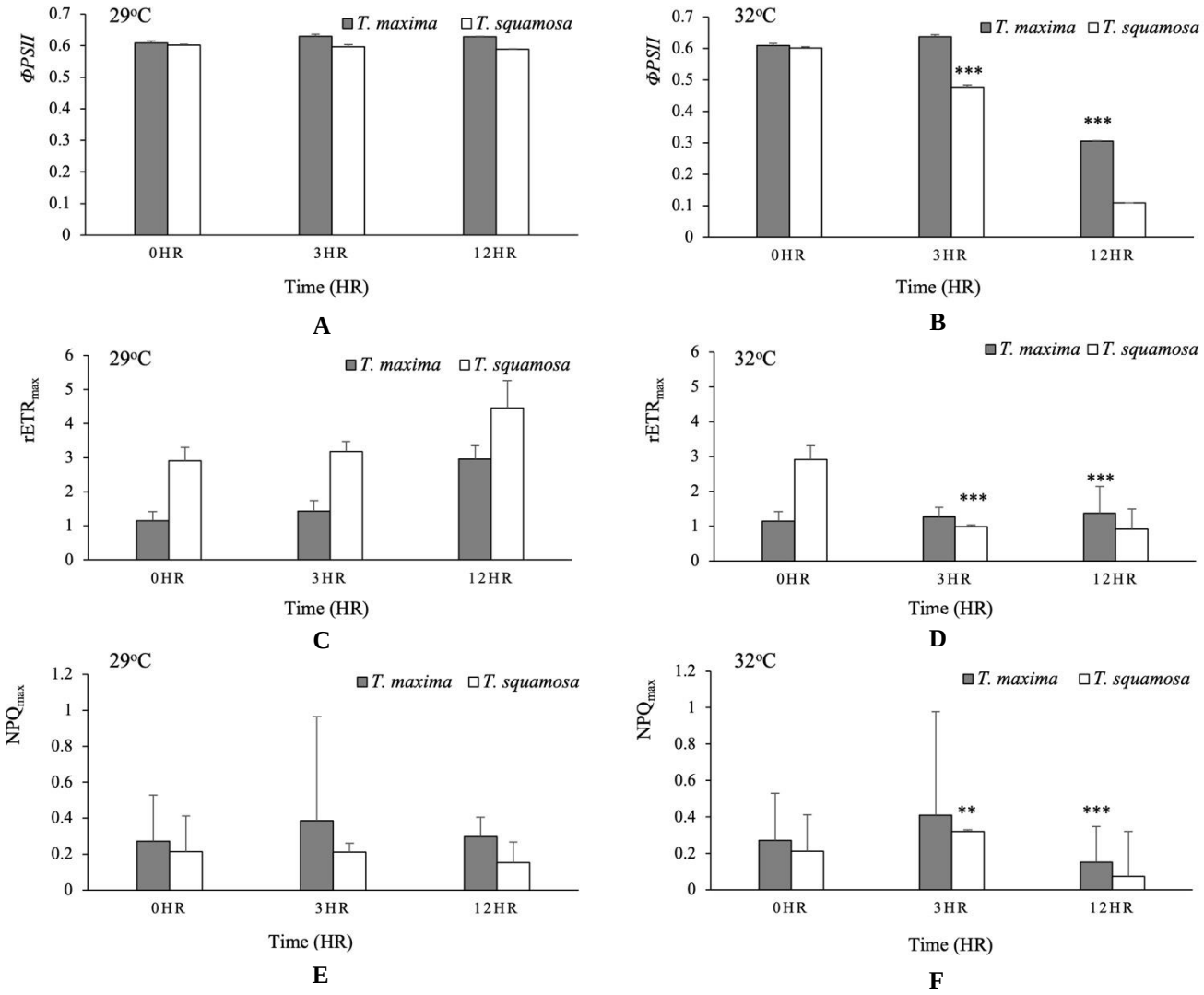

Figure 3. Φ_{PSII}, $rETR_{max}$ and NPQ_{max} responses of *T. maxima* and *T. squamosa* at 29°C and 32°C for 0, 3 and 12 hours. Bars represent Mean + SD (n=3)

Table 1. Two-way ANOVA on the effect of giant clam species on the photo-physiology responses (Φ_{PSII}, $rETR_{max}$ and NPQ_{max}) to two temperatures namely 29°C and 32°C over two time points 3 and 12 hours (taking in consideration that at time 0 there will be no significant change recorded) . $p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = ***

Parameters	Variation	df	MS	F	*p*-value
Φ_{PSII}	Species	1	0.020	80.59	**
	Temperature	1	0.001	200.15	***
	Species x Temperature	1	0.089	30.25	*
$rETR_{max}$	Species	1	0.039	168.25	**
	Temperature	1	0.068	226.39	**
	Species x Temperature	1	0.005	236.01	*
NPQ_{max}	Species	1	0.090	14.23	**
	Temperature	1	0.074	32.22	***
	Species x Temperature	1	0.062	369.55	*

Discussion

Thermal stress is known to be one of the leading causes of bleaching. Similar to corals, giant clams have been largely affected by global ocean warming (Watson and Neo 2021). An increase in seawater temperature can also negatively affect giant clams since they also host and strongly depend on symbiotic Symbiodiniaceae to survive (Blidberg et al. 2000). The sea surface temperature (SST) of the Republic of Mauritius waters is known to vary between 23°C (in winter) to 30°C (in summer) (Bhagooli

and Kaullysing 2019). The temperature of 29ºC was, therefore, chosen to be closest to the normal summer maximal temperature in Mauritian waters. It was observed that both species of *Tridacna* could tolerate the temperature without being much affected in terms of their photo-physiological responses. The temperature of 32ºC, that is 3ºC above the normal temperature on a normal summer day (29ºC), was used to mimic the condition of the *El Niño* or an event of an extreme increase in water temperature as has been recorded previously in the waters of Mauritius (Bhagooli and Taleb-Hossenkhan 2012; Mattan-Moorgawa et al. 2012). The results of this study corroborate those of other studies such as Blidberg et al. (2000) who investigated the physiological responses of three species of giant clams by exposing them to different elevated temperature stress for 24 hours. It was observed that both respiration and primary production decreased when heat stress was increased. Junchompoo et al. (2013) reported altered physiological conditions of giant clams when seawater temperature fluctuated between 28.8 - 31.1ºC in Thailand coastal waters. The first bleaching event, characterised by loss of the micro-algal symbionts, was recorded with 60% of individuals showing faded coloration, 30% partly bleached and 10% bleached completely. Moreover, in response to a sustained increase in temperature, bleaching became more severe with 90% completely bleached, 8% partly bleached and just 2% remaining with faded colouring. Exposure to temperatures over 30ºC for longer than two weeks has been shown to result in the expulsion of the symbiotic living Symbiodiniaceae thus, depriving the giant clams of carbon (Junchompoo et al. 2013). These results concur with the results obtained from this study where the giant clam *T. maxima* showed sign of mild bleaching following 12 hours of consequent thermal stress, while *T. squamosa* showed sign of disintegration as compared to that of Brahmi et al. (2022) which demonstrated that at 30.7ºC, the symbiont's photosynthetic yield of *T. maxima* was highly impacted.

Dubousquet et al. (2016) demonstrated that when the temperature reached 31ºC and 32ºC, the giant clam *T. maxima*'s symbionts died and were degraded under thermal stress. They further put forward that the degraded symbiont's cells were used as an additional energy source for *T. maxima* to sustain the stress being encountered. It has been suggested in other studies that just like in some nudibranch (Burghardt and Wägele 2014; Norton et al. 1992; Soo and Todd 2014), sea anemones and corals (Dunn et al. 2004; Strychar and Sammarco 2009), the Symbiodiniaceae within giant clams are believed to be cultured by the host as a source of nutrition and energy supply as and when required by the host. Dubousquet et al. (2016) suggested that the degradation of Symbiodiniaceae cells observed during elevated temperature might offer *T. maxima* an alternative and rapid source of food which would delay the depletion of the lipid storage which goes in consistency with the energy budget model depicted by Brown et al. (2004) and Anthony et al. (2009) in their studies. However, this study revealed that, unlike *T. maxima*, *T. squamosa* was more susceptible to degradation after an elevated temperature stress. The NPQ_{max} which is the protective mechanism (Slavova et al. 2016) showed rapid degradation. This may suggest that as compared to *T. maxima*, *T. squamosa* may not be able to use the degraded Symbiodiniaceae as an alternative and rapid source of food, an adaptive mechanism that may help the giant clams to delay the mortality process due to thermal stresses.

Genetic clades of Symbiodiniaceae have unique physiological characteristics (Rowan et al. 1997; Tchemov et al. 2004; Sampayo et al. 2008; Bhagooli 2009, 2010; Ghoora et al. 2018; Mattan-Moorgawa et al. 2020) which may play a big role in the host's survival affinity and its susceptibility to environmental stresses and changes (Baker 2001). Giant clams are known to host Symbiodiniaceae of clades A, C and D only or a mixture of these clades (Baillie et al. 2000 a,b; DeBoer et al. 2012). The choice of the clades and their composition is highly dependent on their surrounding environment such as seawater temperature such that giant clams that hosted the Symbiodiniaceae of clades C and D were known to be in area where mean seawater temperature was elevated (DeBoer et al. 2012). Though, Rowan (2004) showed that a temperature of 32°C decreased Φ_{PSII} in clade C, whereas clade D maintained an increased Φ_{PSII} which concurred with this study. One may be tempted to suggest that the survival of giant clams would be highly dependent on its Symbiodinaceae. Since the clade identity wasn't investigated in this study, further study is required in this direction.

The findings of this study showed that the giant clam *T. maxima* is more resistant to thermal stress as compared to *T. squamosa*. This could be explained by the fact that *T. maxima* may have the ability to delay its mortality process caused by thermal stress as compared to *T. squamosa*. However, further in-depth studies are warranted in this area to determine the survivorship and tolerance of giant clams to global warming along with more advanced studies on their Symbiodinaceae species susceptibility.

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Variations in photo-physiological responses of shaded and non-shaded mangrove, *Rhizophora mucronata* tree parts from Mauritius Island, western Indian Ocean

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Abstract. *Kaullysing D, Jogee SY, Mundil SP, Soondur M, Gopeechund A, Ricot M, Jeetun S, Chinta T, Chockalingum J, Mungur D, Kowal B, Kristnama L, Gunness V, Balgobin A, Fakun ZR, Munbodhe V, Nohur MB, Ramdhun D, Ramsurrun LK, Rase S, Seetohul TK, Mattan-Moorgawa S, Ramah S, Bhagooli R. 2023. Variations in photo-physiological responses of shaded and non-shaded mangrove,* Rhizophora mucronata *tree parts from Mauritius Island, western Indian Ocean. Indo Pac J Ocean Life 7: 71-78.* This study assessed and compared the photo-physiological responses of the tree parts of juvenile and adult mangrove, *Rhizophora mucronata*, under shaded and non-shaded conditions in the northern coast of Mauritius Island. Chlorophyll *a* fluorescence of mature (dark) leaves, young and mature propagules, lichen, buds, and sepal of adult *R. mucronata* trees, and of mature and young (pale) leaves of juveniles under natural shaded and non-shaded conditions was measured using a field-portable Diving Pulse-Amplitude-Modulated (D-PAM) fluorometer. Commonly used chlorophyll fluorescence parameters such as F_v/F_m, $rETR_{max}$, NPQ_{max} were calculated. The tree parts of adult and juvenile *R. mucronata* showed considerable variations in their photosynthetic responses. F_v/F_m of adult tree leaves was 30% higher in shaded condition as compared to non-shaded condition. The combined effect of mangrove tree parts and conditions (shaded; non-shaded) resulted in significant differences in mean values of F_v/F_m (three-way ANOVA, $p<0.001$). Leaves of adult trees had 52% higher $rETR_{max}$ in shaded condition. While a significant difference ($p<0.001$) was noted in the mean $rETR_{max}$ values of various tree parts, the shaded and non-shaded conditions did not have any significant effect on $rETR_{max}$ ($p>0.05$). Non-shaded parts of *R. mucronata*, including the leaves, exhibited higher NPQ_{max} values as compared to shaded conditions. Mean NPQ_{max} varied significantly among mangrove parts ($p<0.001$), between tree stage ($p<0.001$) and between conditions ($p<0.05$). These findings revealed differences in the photosynthetic activities of various mangrove parts of juvenile and adult trees under shaded and non-shaded conditions, a first attempt for the tropical island of Mauritius.

Keywords: Chlorophyll *a*, mangrove, Mauritius, photo-physiology, shaded and non-shaded conditions

INTRODUCTION

Mangroves are unique and complex vascular plants inhabiting tropical and sub-tropical coasts (Cheeseman et al. 1997; Polidoro et al. 2010). An estimated 14.79 million hectares of mangrove forests are present in 113 countries around the world (FAO and UNEP 2020). Mangrove plants of the world consist of approximately 70 species of trees and shrubs (Spalding and Leal 2021). In Mauritius, only two species of mangroves occur, namely, *Bruguiera gymnorrhiza* and *Rhizophora mucronata* (Appadoo 2003). However, only species belonging to the genera *Avicennia, Lumnitzera, Bruguiera, Ceriops, Kandelia, Rhizophora* and *Sonneratia*, and the species *Nypa fruticans* and *Laguncularia racemosa* are considered as "true mangroves" and are the main components of mangrove forests worldwide (Tomlinson 2016 cited in Quadros and Zimmer 2017). This implies that they are woody plants, facultative or obligate halophytes inhabiting the intertidal region and are not found in terrestrial communities (Komiyama et al. 2008; Quadros and Zimmer 2017). Mangrove trees are usually associated with other organisms such as lichens, microbes, other plants, and animals (Kathiresan and Bingham 2001), and mangrove forests are composed of both autochthonous (local) and allochthonous (external) organic carbon, as evidenced by the analysis of sediment cores (Suello et al. 2022).

Tang et al. (2018) used Geographic Information System (GIS)-based geospatial analysis and high-performance parallel computing and estimated the total area, biomass (including above- and below-ground), and associated carbon stock of global mangroves as 130,420 km^2, 1.908

Pg, and 0.725 Pg C, respectively, for the year 2000. The averaged above-ground biomass density of global mangroves was estimated as 146.3 Mg ha^{-1} for the same year (Tang et al. 2018). From 1990 to 2020, mangrove area has decreased by 1.04 million hectares (FAO and UNEP 2020), contributing significantly to loss of carbon stocks (Monga et al. 2022) and associated ecosystem services (Goldberg et al. 2020). It is estimated that mangrove deforestation results in emissions of 0.02-0.12 Pg C per year, which is as much as around 10% of emissions from deforestation globally, despite accounting for merely 0.7% of tropical forest area (Donato et al. 2011). This highlights the importance of protecting mangrove forests from destructive anthropogenic activities, as well as natural causes of degradation.

Mangroves are physiologically interesting as prospective models for stress tolerance (Cheeseman et al. 1997) as they have adapted to live in extreme conditions such as high temperature, anaerobic soil, tidal influx, and high salinity (Kathiresan and Bingham 2001). A convenient way of measuring stress in mangroves is by estimating their photosynthetic activities through the measurement of the chlorophyll *a* fluorescence. Mangroves perform C3 photosynthesis and the photosynthetic activity of mangroves differs from one species to another (Basak et al. 1996) depending on several external environmental factors such as salinity level, ambient temperature, and ambient light levels (Cheeseman et al. 1991; Cheeseman 1994). Photosynthetic pigments such as chlorophylls are also found in the propagules (Duke and Watkinson 2002), stems and branches (Saveyn et al. 2010; Schmitz et al. 2012) of mangroves. Therefore, different parts of mangrove trees are capable of performing photosynthesis which is a key process for their survival and sustainability of the ecosystem as a whole. Globally, numerous studies have investigated photo-physiological responses of mangrove leaves (Rovai et al. 2013; Naidoo et al. 2014), while fewer studies investigated that of the stem (Schmitz et al. 2012). The photosynthetic activity of other parts such as the pegs, flowers and epiphytic lichens are yet to be thoroughly understood. It is noteworthy that plant parts in other marine seaplants have been studied. For instance, the photosynthetic performance was found to be variable among the leaf, transition part between leaf and stem (transit), the stem of the seagrass *Thalassodendron ciliatum*, and between the frond and the stolon of the macroalga *Caulerpa cupressoides* (Bhagooli et al. 2021a).

Mangrove afforestation programmes in Mauritius have enabled the declining area to increase from 45 ha in the year 1980 to 145 ha in 2013 (Bosire et al. 2016). However, such programmes are carried out without an assessment of the photo-physiological responses of mangroves to the local conditions and climate. Furthermore, though there are several scientific studies related to mangrove areas around Mauritius (Sadally et al. 2016; Armance et al. 2019; Soondur et al. 2020, 2021), no information is available about the differential photo-physiological responses in the different parts of adult and juvenile mangrove. Thus, with the intention of contributing to providing new and additional information on tropical mangroves of Mauritius, this study assessed and compared the photo-physiological responses of the photosynthetic tree parts of juvenile and adult *R. mucronata*, the dominant species of mangroves in the tropical island of Mauritius, under shaded and non-shaded conditions.

MATERIALS AND METHODS

Study site

The study was conducted in a mangrove patch at Poudre d'Or situated in the north-east coast of Mauritius Island (Figures 1A,B) in September 2020. Juvenile and adult trees of *R. mucronata* extended along the intertidal zone within the barachois and seedlings were mostly found under the canopy of the adult trees. Saplings and some more developed seedlings were found along the supratidal zone (Figure 1C). For the purpose of this study, mangrove trees taller than 3 m were considered as adults (Clarisse et al. 2016), while juvenile trees were defined as those individuals with a height greater than 0.3 m but smaller than 3 m (Osland et al. 2020). Adult and juvenile trees/parts in the non-shaded condition were those that were exposed continuously to sunlight over maximum duration of the day. Shaded juvenile trees were sheltered from direct sunlight under the adult trees over maximum duration of the day, while shaded adult trees were constantly protected from direct sunlight among other taller adult trees.

Measurement of photosynthetic activity

Measurement of photosystem II fluorescence represents the best approach to estimating the photosynthetic activity of mangroves. The use of Pulse Amplitude Modulated (PAM) fluorometry (Genty et al. 1990; Bhagooli et al. 2021b) as a non-invasive method is well suited for studying the photo-physiology of photosynthetic organisms, including mangroves (Falqueto et al. 2008). During the present study, chlorophyll *a* fluorescence was measured using a field-portable Diving-PAM fluorometer (Heinz Walz GmbH, Effeltrich, Germany). Following dark adaptation of 30 minutes, measurements were taken between 1130 to 1430 hrs on three random samples/individuals (triplicate) of mature (dark) leaves, young and mature propagules, lichen, buds, and sepal of adult *R. mucronata* trees, and of mature and young (pale) leaves of juveniles under natural shaded and non-shaded conditions in the mangrove stand at the study site. The commonly used fluorescence parameters F_v/F_m (maximum photochemical efficiency of photosystem II), $rETR_{max}$ (maximum relative electron transport rate), NPQ_{max} (maximum non-photochemical quenching), as well as α (initial slope of the Rapid Light Curve before the onset of saturation), β (slope of the RLC after saturation) and I_k (minimum saturating irradiance) were calculated from the fluorescence parameters measured (Bhagooli et al. 2021b). $rETR_{max}$, NPQ_{max}, α and β values were obtained after fitting curves to the RLCs through Sigmaplot (Version 12.0).

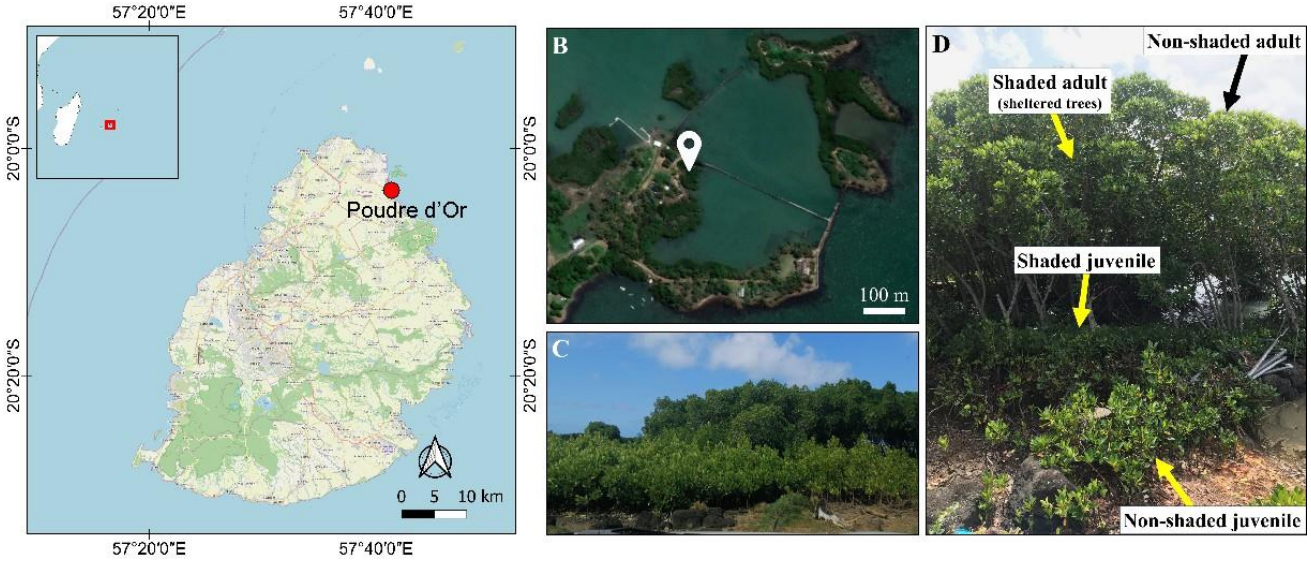

Figure 1. A. Map of Mauritius showing the study site located in the north-east of Mauritius, B. Satellite image of Poudre d'Or barachois with mangrove patch indicated by a white pin (Source: Google Earth Pro 2022), C. The study site with a mangrove patch composed of adult (background) and juvenile (foreground) trees, D. Adult mangrove trees and juveniles in shaded and non-shaded conditions

Measurement of physico-chemical parameters

To quantify the characteristics of the shaded and non-shaded conditions, air temperature and light intensity levels were recorded in situ using data loggers (HOBO, Pendant) which were affixed with cable ties on shaded and non-shaded adult and juvenile trees. Seawater salinity was also measured using a salinity logger. Measurements were taken at an interval of 15 minutes over the study period.

Statistical analyses

The normality of the data was determined by the Shapiro-Wilk test. Non-normal data were arcsine (sqrt) transformed to meet the assumptions of normality. A three-way Analysis of Variance (ANOVA) was used to determine differences in mean F_v/F_m, $rETR_{max}$, NPQ_{max}, α, β and I_k values of different parts of adult and juvenile mangroves, including in the epiphytic lichens under shaded and non-shaded conditions, followed by LSD post hoc test. All statistical analyses were conducted using SPSS statistical software (PASW Statistics 18.0.0).

RESULTS AND DISCUSSION

Temperature and light intensity variations

In Mauritius, the annual mean temperature in summer is 24.7°C (Bhagooli and Kaullysing 2019). During the study period, air temperature and light intensity varied between 23.29-24.06°C (Figure 2A) and 3444.5-11022.3 Lux (Figure 2B), respectively where the shaded adult mangrove trees were located. At the non-shaded or exposed zone of adult mangrove trees, air temperature and light level ranged between 27.08-31.88°C (Figure 2A) and 33066.9-121245.2 Lux (Figure 2B), respectively. At the shaded juvenile mangrove patch located under the adult mangrove canopy, air temperature and light intensity varied between 23.97-24.64°C (Figure 2C) and 4822.3-18600.1 Lux (Figure 2D), respectively. In contrast, non-shaded juvenile trees experience an air temperature and light intensity between 28.45-34.80°C (Figure 2C) and 46844.8-286579.7 Lux (Figure 2D), respectively. Non-shaded adult *R. mucronata* trees received light intensity almost 11-fold higher than shaded ones, while non-shaded juvenile trees received light intensity more than 15-fold higher than shaded ones. Seawater salinity varied between 34.5-35.5 PSU during the study period.

Photo-physiology of *Rhizophora mucronata*

The tree parts of adult and juvenile *R. mucronata* showed considerable variations in their photosynthetic responses. The leaves of adult trees had the highest F_v/F_m followed by the young propagules, both under shaded and non-shaded conditions (Figure 3A). A significant difference was noted in F_v/F_m values under shaded and non-shaded conditions (three-way ANOVA, $p<0.05$). F_v/F_m of adult leaves, juvenile leaves (young and mature), and propagules (young and mature) were higher under shaded condition. F_v/F_m of adult tree leaves was 30% higher in shaded condition as compared to non-shaded condition. In contrast, the lichen associated with the bark of the mangrove trees showed higher F_v/F_m in non-shaded condition. Buds and sepal of adult trees, measured only under non-shaded condition, also exhibited comparable F_v/F_m to the other tree parts under the same condition. The combined effect of mangrove tree parts and condition (shaded; non-shaded) resulted in significant differences in mean values of F_v/F_m ($p<0.001$; Table 1). $rETR_{max}$ was higher in young leaves of juvenile *R. mucronata* in shaded condition, while the mature leaves had higher maximum relative electron transport rate in non-shaded condition. Leaves of adult trees had 52% higher $rETR_{max}$ in shaded condition. Young and mature propagules and lichen on the bark had lower $rETR_{max}$ in shaded condition (Figure 3B). While a significant difference ($p<0.001$) was noted in the

mean $rETR_{max}$ values of various tree parts, the shaded and non-shaded conditions did not have any significant effect on $rETR_{max}$ ($p>0.05$; Table 1). Overall, mean NPQ_{max} varied significantly among mangrove parts ($p<0.001$), between tree stage ($p<0.001$) and between conditions ($p<0.05$). In general, non-shaded parts of *R. mucronata* exhibited higher NPQ_{max} values as compared to shaded conditions (Figure 3C). No significant differences in mean α and ß values were noted among the mangrove parts, however, there were significant differences in α between juvenile and adult *R. mucronata* ($p<0.05$; Figure 3D). No significant differences were noted in ß under any treatment ($p<0.05$; Figure 3E). I_k values were lower for leaves of juvenile *R. mucronata* and higher for leaves of adult trees in shaded condition (Figure 3F) with significant differences between mangrove parts and tree stages ($p<0.05$).

Discussion

Being one of the most productive ecosystems on the planet, mangrove forests sequester a significant amount of carbon dioxide, thus, representing a globally important carbon sink (Sanderman et al. 2018). A thorough understanding of mangrove physiological conditions, community structure, and ecosystem processes is crucial to efficiently channel efforts toward the conservation of this highly threatened ecosystem, especially in data-deficient regions such as the western Indian Ocean, including the tropical island of Mauritius which relies heavily on its surrounding natural coastal and marine ecosystems for the protection of its coastal zones. This study revealed the photosynthetic activity of various mangrove parts of juvenile and adult trees under shaded and non-shaded conditions.

Table 1. Three-way ANOVA for effects of different mangrove parts, tree stage (juvenile and adult) and condition (shaded and non-shaded) on the photo-physiological parameters (F_v/F_m, $rETR_{max}$, NPQ_{max}, α, ß and I_k). * - $p<0.05$, ** - $p<0.01$, *** - $p<0.001$

Dependent variable	Source of variation	*df*	*MS*	*F*	*p value*	
F_v/F_m	Mangrove part	5	0.132	21.549	0.000	***
	Tree stage	1	0.001	0.132	0.719	NS
	Condition	1	0.134	21.929	0.000	***
	Mangrove part * Tree stage	1	0.001	0.082	0.777	NS
	Mangrove part * Condition	3	0.062	10.169	0.000	***
	Tree stage * Condition	1	5.140E-7	0.000	0.993	NS
	Mangrove part * Tree stage * Condition	1	0.006	1.056	0.313	NS
$rETR_{max}$	Mangrove part	5	0.084	10.065	0.000	***
	Tree stage	1	0.012	1.385	0.250	NS
	Condition	1	0.001	0.154	0.698	NS
	Mangrove part * Tree stage	1	0.057	6.830	0.014	*
	Mangrove part * Condition	3	0.024	3.623	0.026	*
	Tree stage * Condition	1	0.030	5.232	0.030	*
	Mangrove part * Tree stage * Condition	1	0.028	3.349	0.078	NS
NPQ_{max}	Mangrove part	5	0.066	37.187	0.000	***
	Tree stage	1	0.006	3.467	0.000	***
	Condition	1	0.024	13.553	0.001	**
	Mangrove part * Tree stage	1	1.913E-6	0.001	0.974	NS
	Mangrove part * Condition	3	0.013	7.491	0.001	**
	Tree stage * Condition	1	0.000	0.147	0.704	NS
	Mangrove part * Tree stage * Condition	1	0.007	3.796	0.062	NS
α	Mangrove part	5	0.004	1.676	0.174	NS
	Tree stage	1	0.012	4.755	0.038	*
	Condition	1	0.005	2.204	0.149	NS
	Mangrove part * Tree stage	1	0.026	10.388	0.003	**
	Mangrove part * Condition	3	0.004	1.796	0.172	NS
	Tree stage * Condition	1	0.001	0.463	0.502	NS
	Mangrove part * Tree stage * Condition	1	0.003	1.174	0.288	NS
ß	Mangrove part	5	0.016	1.305	0.291	NS
	Tree stage	1	0.011	0.914	0.347	NS
	Condition	1	0.012	0.993	0.328	NS
	Mangrove part * Tree stage	1	0.028	2.248	0.145	NS
	Mangrove part * Condition	3	0.034	2.790	0.060	NS
	Tree stage * Condition	1	0.017	1.364	0.253	NS
	Mangrove part * Tree stage * Condition	1	0.001	0.097	0.758	NS
I_k	Mangrove part	5	0.033	3.503	0.014	*
	Tree stage	1	0.057	6.088	0.020	*
	Condition	1	0.024	2.589	0.119	NS
	Mangrove part * Tree stage	1	0.061	6.536	0.017	*
	Mangrove part * Condition	3	0.106	11.336	0.000	***
	Tree stage * Condition	1	0.261	27.895	0.000	***
	Mangrove part * Tree stage * Condition	1	0.006	0.653	0.426	NS

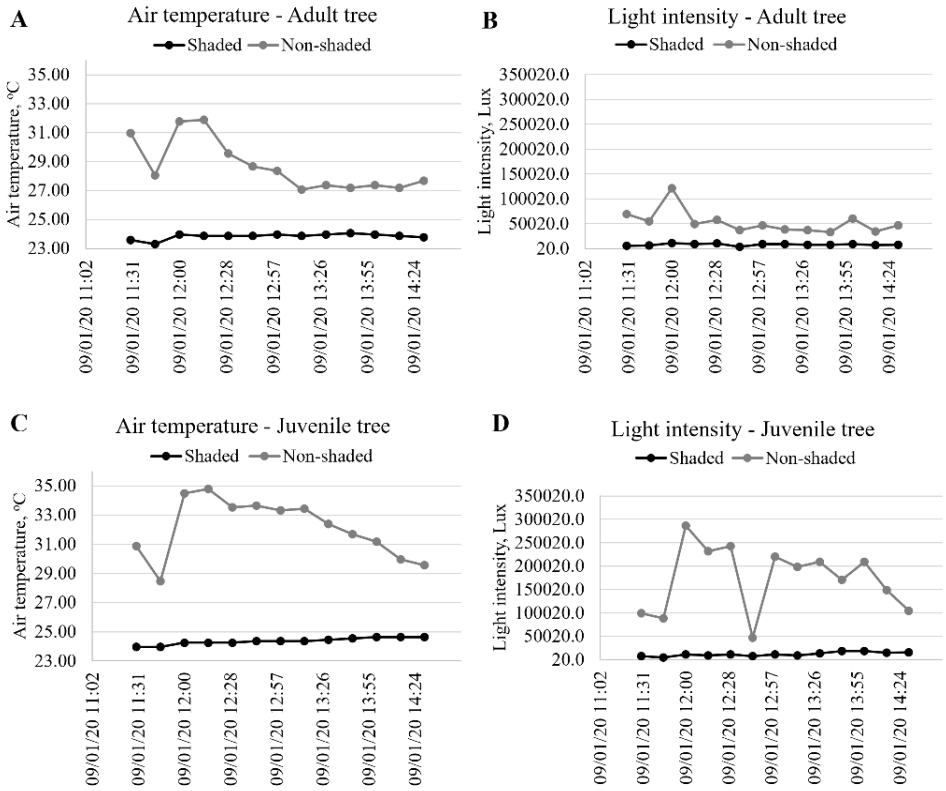

Figure 2. Variations in air temperature and light intensity for adult (A, B) and juvenile (C, D) *R. mucronata* at shaded and non-shaded zones within the studied mangrove patch

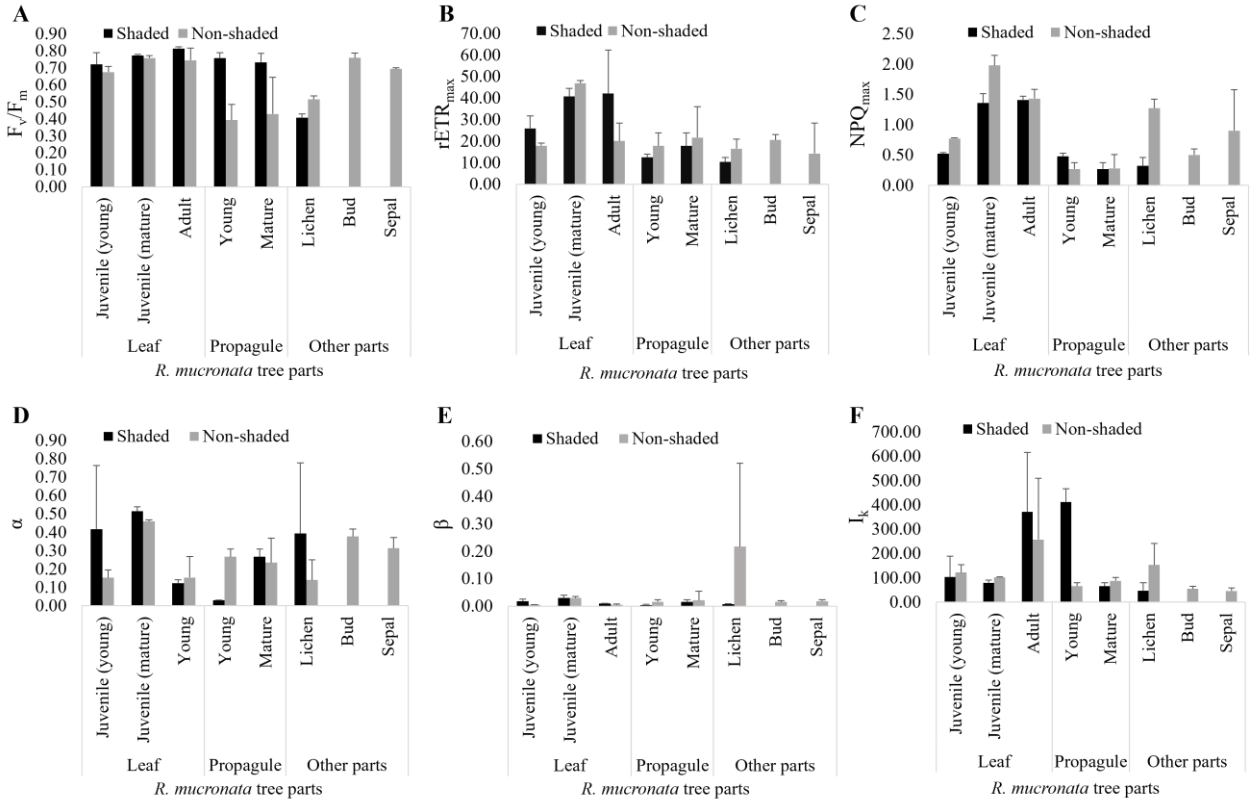

Figure 3. A. F_v/F_m, B. $rETR_{max}$, C. NPQ_{max}, D. α, E. β and F. I_k of the different parts of juvenile and adult *R. mucronata* trees under shaded and non-shaded conditions. Bars represent mean ± standard deviation

Mangroves are physiologically interesting plants thriving in a harsh environment where conditions are expected to reduce their photosynthetic capacity through photoinhibition. In the natural habitat, mangroves receive high luminosity which is much more than the saturation point of photosynthesis, indicating that mangrove leaves often receive a large amount of excess light energy and may be prone to photo-inhibition (Cheeseman et al. 1994; Kitao et al. 2003; Attiwill and Clough 2018). Mangrove photosynthesis can reach light saturation under the incident photosynthetic photon flux density of 40% sunlight (800-1000 µmol photon.m^{-2}.s^{-1}) or lower in harsh tropical intertidal environmental conditions (Andrews et al. 1984; Carter et al. 1990; Cheeseman 1994).

Despite the extreme conditions, most species of mangroves have adapted their leaves and maintain this main active photosynthetic organ under optimum conditions to reduce photoinhibition and to sustain productivity. The shaded condition of the mangroves at Poudre d'Or led to the availability of 11- to 15-fold lower light intensity for the photosynthesizing mangrove parts, going as low as 3444.5 Lux (64 µmol photon.m^{-2}.s^{-1}). The mangrove parts exposed to higher intensities of light had lower photosynthetic activities as compared to the shaded parts, as evidenced by higher values of F_v/F_m under non-shaded conditions. Low light conditions may not necessarily imply reduced photosynthetic activities.

Additionally, the photosynthetic apparatus of mangroves are also adapted to recover rapidly from high light levels such that F_v/F_m reaches 0.8 shortly after sunset without occurrence of chronic photoinhibition and quantum efficiencies of PSII were high up to 500 µmol quanta.m^{-2}.s^{-1}, indicative of well-protected PSII activity, as demonstrated by Cheeseman (1994). In contrast to the findings of this study, Björkman et al. (1988) reported a large decrease in F_v/F_m when leaves of a number of mangroves were directly illuminated in the field, while Cheeseman et al. (1991) reported no evidence of photoinhibition in exposed leaves of *Bruguiera parviflora* under natural illumination. This aspect of the metabolism of mangroves might be crucial for photosynthesis.

Leaves of *R. mucronata* when exposed to high illumination (non-shaded condition) at Poudre d'Or exhibited higher NPQ_{max}, indicating the dissipation of excess radiation through non-damaging heat emissions (Bhagooli et al. 2021b). NPQ is an important photo-protective mechanism of plants in response to the high light irradiance damage, which quenches excess energy absorbed as Photosynthetically Active Radiation (PAR) and converts and dissipates it as heat to avoid the harmful effects of excessive photon absorption (Murchie and Niyogi 2011; Esteban et al. 2013). Similar observations have been made for *Abies alba* (Pinaceae, Coniferales) by Dörken and Lepetit (2018) whereby sun leaves had higher NPQ as compared to shade leaves.

While eco-physiological responses of mangroves to excess luminosity vary between different species and may considerably affect the survival rate and spatial distribution of the mangroves, the difference in their photosynthetic capacity is closely related to salinity also (Naidoo and Von Willert 1995; Naidoo et al. 2001; Panda et al. 2006; Dittmann et al. 2022; Wang et al. 2022). A decrease may be noted in stomatal conductance when mangrove ecosystems are exposed to high illumination and high salinity conditions, resulting in reduced CO_2 fixation to generate excess energy. Chen et al. (2022) demonstrated that *Kandelia obovata* and *Rhizophora stylosa* when treated with 30 ppt salinity exhibited lower photosynthetic rate but higher NPQ and heat quenching values, followed by increases in the excess energy and photoprotective effects. In the present study, salinity did not vary considerably. The results of this study suggest remarkable difference between the photosynthetic activities of various mangrove parts of juvenile and adult trees under shaded and non-shaded conditions at a tropical site like Mauritius. However, it is important to also consider the effect of salinity and other parameters on the photosynthesis of mangroves. Therefore, further studies are warranted to assess the diurnal variations in fluorescence parameters under the effect of varying salinities with different tidal levels under field conditions.

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Antioxidant and photo-physiological acclimatisation in tropical macroalgae at sites with distinct nutrient levels

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Abstract. *Gopeechund A, Bhagooli R, Neergheen VS, Bahorun T. 2023. Antioxidant and photo-physiological acclimatisation in tropical macroalgae at sites with distinct nutrient levels. Indo Pac J Ocean Life 7: 79-90.* The antioxidant efficacy, Total Phenolic Contents (TPC) and Total Flavonoid Contents (TFC) were investigated at Gis Gris with Lower Nutrient levels (LN site) and Bain boeuf with Higher Nutrient levels (HN site) around Mauritius Island. A field-based 7-days transplantation manipulation of *Turbinaria ornata*, *Gracilaria salicornia* and *Sargassum obovatum* between HN and LN sites was conducted to test the impact of different nutrient conditions on their physiology. All the species had lower antioxidant efficacies, TPC and TFC at LN compared to HN site. The glutathione peroxidase and catalase were more sensitive at reflecting the lower oxidative stress at LN site and acclimatisation to oxidative stress occurring at the HN site. ETRmax and α were significantly higher in all species at HN site. The quantum yield Fv/Fm increased in *S. obovatum* only, after 7 days of transplantation at HN site, while, *T. ornata* and *G. salicornia* had similar adaptability to photo-physiological stress at both sites. NPQmax decreased in *S. obovatum* but increased in *T. ornata* transplanted to HN site, indicating increased photo-protection at HN site. Lagoons with higher nutrient levels may enhance macroalgal capacity to deal with oxidative stress, thus lowering the need for further photo-protection.

Keywords: Antioxidant efficacy, enzymatic antioxidant, nutrient-dependent variation, phenolic contents, photo-physiology

INTRODUCTION

Most shallow ocean areas receive significant amounts of human-induced chemical inputs and natural environmental conditions (Eklund and Kautsky 2003; Parida and Das 2005; Helmuth et al. 2006; Kannan and Krishnamoorthy 2006). Marine organisms' interaction with pollutants impacts their physiology (Brierley and Kingsford 2009; Dailianis 2010; Morais et al. 2012). Macroalgae may respond to altered environmental conditions in marine areas influenced by human activities by changing their levels of phenolic compounds and antioxidant activities (Orbea et al. 2002; Cunha et al. 2005; Nimptsch et al. 2005; Connan et al. 2006; Scania and Chasani 2021). Collen and Davison (1999) highlighted the exposure of *Fucus* spp. to desiccation or freezing stress increased Reactive Oxygen Species (ROS) production. Increased levels of the enzymatic antioxidant glutathione in the green seaweed *Ulva* sp. correlated with increased levels of dissolved inorganic nitrogen levels (Pereira et al. 2009). Antioxidants have thus been proposed as biomarkers of contaminant-mediated oxidative stress in various marine organisms (Cossu et al. 1997). (Cossu et al. 1997; Gopeechund et al. 2020). However, studies on macroalgae and seagrass antioxidants (Somanah et al. 2012; Ramah et al. 2014; Narrain et al. 2023) and photophysiology (Bhagooli et al. 2021a; Narrain et al. 2023) are limited in the Mauritian waters.

Light plays a key role in triggering different types of stresses in marine organisms. Solar radiation, more specifically short wavelengths (UVB, 280-315 nm) can alter photo-physiological processes in plants, including protective responses (Bischof et al. 2006), DNA damage (Pakker et al. 2000; van de Poll et al. 2001) and growth (Aguilera et al. 2002). Changes in light levels are known to enhance ROS production, for e.g., the production of superoxide increased at photosystem I in the diatom *Thalassia weissflogii* during photosynthesis correlated with changes in the level of light (Milne et al. 2009). There is evidence of phenolic compounds helping in photo-protection. For instance, coumarins in green macroalgae like *Caulerpa* sp. and *Dasycladus* sp. help prevent radiation damage with their high UV absorption properties (Pérez-Rodríguez et al. 2003; Bischof et al. 2006). Intertidal macroalgae are speculated to be physically and physiologically adapted to cope with irradiance fluctuations and maintain optimal conditions for physiological processes such as photosynthesis (Davison and Pearson 1996). Photo-physiological adaptations also occur in response to light induced stress, whereby the photosystem activity is reduced due to strong light (Schagerl and Möstl 2011). Pulse Amplitude Modulation (PAM) fluorometry

uses Rapid Light Curves (RLCs) to provide a measure of chlorophyll a fluorescence (Beer et al. 2006; Campbell et al. 2008; Collier et al. 2009; Bhagooli et al. 2021b). RLC-derived parameters such as maximum quantum yield (Fv/Fm), initial slope (α), ETRmax, and NPQ have been used to indicate stress occurring in marine plants, including algae (Le et al. 2006; Bité et al. 2007; Piniak and Brown 2009; Silverstein et al. 2017). It has also been shown that photophysiological parameters vary during transplantation from one site to another, for example, α and ETRmax decreased in seagrass species post-transplantation to a site with higher freezing stress (Collen and Davison 1999). Marine plants such as seagrasses alter their physiology, morphology and growth after transplantation (Zimmerman et al. 1995; Horn et al. 2009). Marine plants' photosynthetic rate may also be affected by transplantation shock, which may show up in characteristics of chlorophyll *a* fluorescence (Lamote and Dunton 2006; Kahn and Durako 2008; Horn et al. 2009).

Limited studies have experimentally determined the variation of photo-physiological and antioxidant parameters of macroalgae under distinct nutrient levels (Delgado and Lapointe 1994; Teichberg et al. 2013; Moussavou et al. 2014). Given the scarcity of studies describing the nutrient-dependent variation of photo-physiological processes, as well as antioxidant activities and secondary metabolites in macroalgal species, this paper addresses these parameters to assess the status of the macroalgae under prevailing different nutrient conditions. An experimental field trial was conducted to test the changes observed.

MATERIALS AND METHODS

Sample collection

Macroalgal species were collected at two lagoons of Mauritius (Figure 1) in May 2015. Bain Boeuf ("19°59'08"S, 57°36'16"E") was characterized as having High Nutrient level (HN) and Gris Gris ("20°31'28"S, 57°32'02"E") Lower Nutrient level (LN) (Figure 1). The satellite images of these two sites from 2009-2016 was also assessed for environmental changes using Google Earth (Figure 2). In May 2016, the red seaweed *Gracilaria salicornia* and the brown seaweeds *Turbinaria ornata* and *Sargassum obovatum* were collected from the HN and LN sites for transplantation experiment.

Transplantation experiment

Vertical transplantation of *T. ornata*, *G. salicornia* and *S. obovatum* was done between LN and HN sites. Three adult specimens of each species were placed in reticulated, semi-permeable plastic bags and attached using ropes on firm naturally collected blocks. After 5 days of exposure period in shallow areas (1-2m deep), the chlorophyll a stress photo-physiology was measured using the D-PAM fluorometer and the phenolic contents and antioxidant levels were determined.

Photo-physiological parameters

The seaweeds were dark-adapted for about 30 min (Maxwell and Johnson 2000; Schagerl and Möstl 2011; Bhagooli et al. 2021b), after which a saturating pulse was emitted using a Diving-PAM fluorometer to measure the fluorescence. Parameters such as α (Photosynthetic efficiency), ETR_{max}, Fv/Fm and NPQ_{max} were determined. ETR was calculated using the equation ETR= E(Photon irradiance of PAR) × Δ F/F'm (effective quantum yield) (Serôdio et al. 2007) using the light absorption factor of seaweed, that is 4.2. An ETR curve was fitted using an empirical equation of Platt et al. (1980) on SigmaPlot 12.0, to determine ETR_{max}. The initial slope α was determined on the ETR curve. NPQ was calculated using $(F_M - F_M')/F_M'$ (Bilger and Bjorkman 1990), following which the NPQ_{max} was determined. The maximum quantum yield at PSII was determined as Fv/Fm = (FM–F_0)/FM (Genty et al. 1989).

Measurement of physico-chemical parameters

The physico-chemical parameters of seawater at the collection sites were recorded in situ using appropriate apparatus during both periods of collection. Dissolved Oxygen (DO), salinity, pH and temperature were measured using a DO meter (Hanna HI 9142), a refractometer (ERMA), a pH meter (Hanna HI 9024C) and a thermometer (Cormac 314), respectively. The concentrations of nitrate and phosphate in sea water were analyzed in the laboratory using cadmium reduction and ascorbic acid methods, respectively (Greenberg et al. 1992). In the cadmium reduction method, 75 mL of NH_4Cl-EDTA solution was added to 25 mL of water sample and poured in a cadmium column, whereby nitrate present was reduced to nitrite. The collected solution and the end of the column was treated with 2.0 mL of color reagent (85% phosphoric acid and 0.0125 g/mL of sulphonamide dissolved in water) and the color developed was read at 543 nm on a spectrophotometer (Genesys™ 10S, Thermo Scientific, USA) against a distilled water reagent blank. The concentration of phosphate was determined by adding $5cm^3$ of mix reagent (15 mL of 40g/L ammonium heptamolybdate, 15 mL of 3.4g/L potassium antimonyltartrate, 50 mL $5NH_2SO_4$, and 30 mL of 17.6 g/L of ascorbic acid) to $50cm^3$ of water sample. After 15 minutes the absorbance was measured at 880 nm on a spectrophotometer, using distilled deionized water as blank.

Macroalgal extract preparation

Extracts were prepared using protocol of Chakraborty et al. (2013), with modifications in the concentration of methanol used. First, 200g of each seaweed species was collected, freeze-dried and macerated in 70% methanol 1:2 w/v. The filtered extracts were concentrated using a rotary evaporator and then freeze dried into powder. The powdered extracts were then prepared and tested for phytochemicals and investigated for antioxidant efficacy.

Phytochemical analyses

Estimation of total phenolic content

The method of (Singleton et al. 2015) was used for the quantification of total phenolic contents with modifications in volume of reagents used. For the quantification of the total phenol content of the extract using a modified method from the study of Singleton and Rossi (1965) was used with slight modifications. Briefly, 50 µL of extract (0.1-20 mg/mL) was added to 50 µL of distilled water followed by 50 µL of Folin-Ciocalteau reagent. After 3 minutes 200 µL of sodium carbonate was added and the reaction mixture incubated at 40.0°C for 40 minutes. The intensity of the blue coloration was read at 685 nm against a blank (microplate reader: Bio Tek®, USA). A calibration curve of Gallic acid was used to estimate the gallic acid equivalent content of phenolics in milligram per millilitre of extract.

Estimation of total flavonoid content

Quantification of total flavonoids in the extract was done (Lamaison and Carnat 1991). 100 µL of extract (0.1-20 mg/mL), and 100 µL aluminum chloride was incubated at 26°C for 5 minutes. The optical density was read at 440 nm against a blank (microplate reader: Bio Tek® USA). A calibration curve of quercetin was used (20-100 mg/mL) to express results in microgram per gram of the extract. The total flavonoid contents expressed in quercetin equivalent/gFDW.

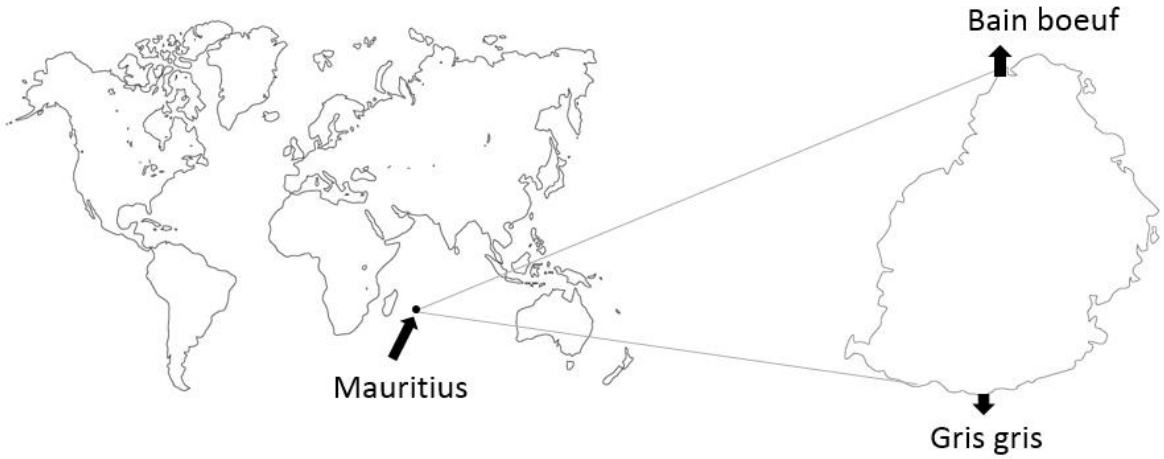

Figure 1. Location of Mauritius in world map and sites of collection in Mauritius: Bain Boeuf and Gris Gris

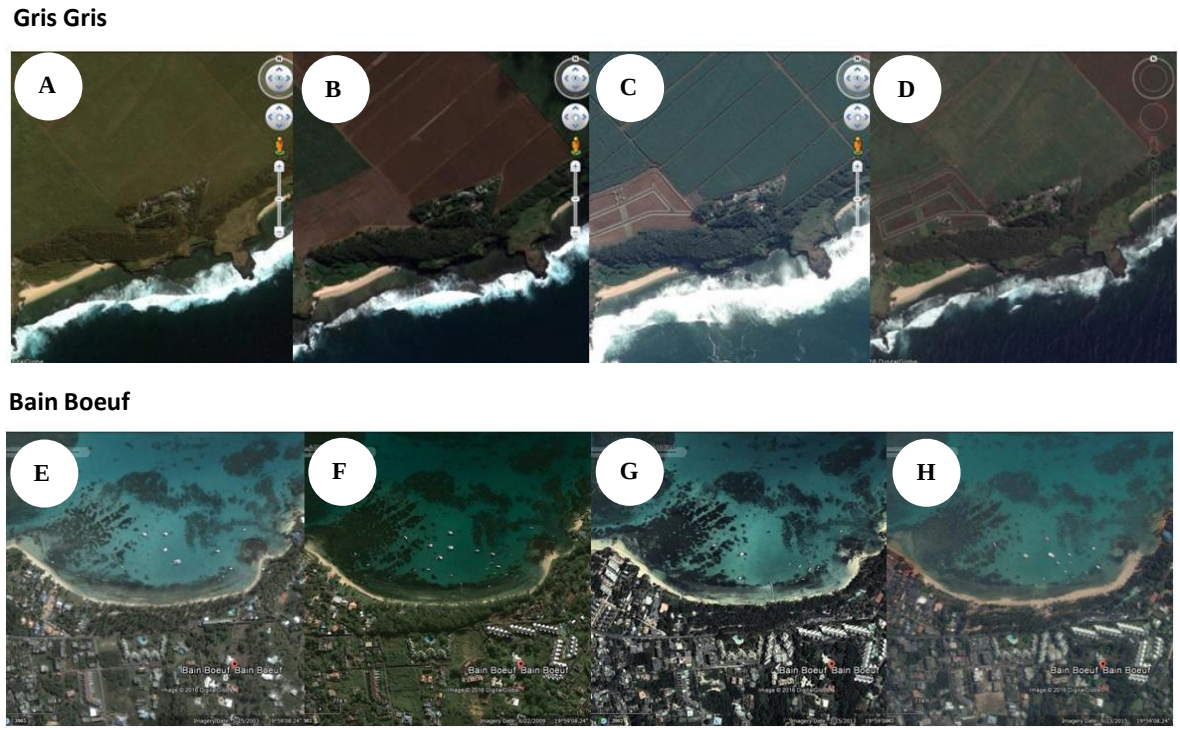

Figure 2. Change in the topography of Gris Gris from 2009-2016 (A-D) and Bain Boeuf from 2003-2015 (E-H). Source: https://www.google.com/earth/

Antioxidant activities

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH radical scavenging assay was used to determine the potential of the extracts to scavenge 2,2-diphenyl-1-picrylhydrazyl radicals (Duan et al. 2007). To 0.1 mL of 2, 2-diphenyl-1-picrylhydrazyl (0.16 mmol/L), 0.1 mL of each extract (0.1-20 mg/mL) was added and the mixture incubated for 30 minutes in darkness at room temperature. The optical density was read at 515 nm (microplate reader: Bio Tek®, USA) and percentage of DPPH radical scavenged was calculated using the formula: DPPH radical Scavenging (%) = $[(A_0 – A_1) / A_0] * 100$. The concentration at which 50 % of the reaction was completed (EC_{50}) was determined. The activities of the species were compared with ascorbic acid, which was used as positive control.

Superoxide scavenging assay

The ability of the extracts to scavenge superoxide anion was determined using the method of Kumar et al. (2011). A total of 1 mL of nitroblue tetrazolium (NBT) solution (156 µM), 1 mL *β*-nicotinamide-adenine dinucleotide, reduced (NADH*;* 200 µM) and 250 µL of each extract (0.1-20 mg/mL) were mixed and the reaction initiated by addition of 100 µL phenazine methosulphate (PMS) solution. The reaction mixture was incubated at 25°C for 30 minutes and the absorbance at 560 nm was measured against a blank (microplate reader: Bio Tek®, USA). The ability of the extracts to scavenge superoxide radicals in the PMS/NADH system was expressed as a percentage using the formula: Scavenging of Superoxide Anion (%) = $[(A_0 – A_1) / A_0] * 100$. Where A_0: absorbance of control (reaction mixture with extract vehicle only) and A_1: absorbance of the sample extract. The concentration at which 50% of the reaction was completed (EC_{50}) was determined. The activities of the species were compared with ascorbic acid, which was used as positive control.

Nitric oxide radical inhibition assay

The nitric oxide radical inhibition assay was used to determine the nitric oxide radical inhibition (Kumar et al. 2008). Greiss Ilosvay reagent was used to quantify nitrite ions produced from the spontaneous oxidation of sodium nitroprusside in aqueous solution. At a final volume of 150 µL, the reaction mixture containing 50 µL sodium nitroprusside (5 mM dissolved in phosphate buffered saline, pH 7) and 50 µL of the extract was incubated at 25°C for 150 minutes. After incubation, 50 µL sulphanilic acid reagent in glacial acetic acid followed by 50 µL N-1-napthylethylenediamine dihydrochloride were mixed and allowed to stand. The absorbance was measured at 546 nm against a blank (microplate reader: Bio Tek®, USA). The scavenging of nitric oxide was calculated using the formula: Scavenging of nitric oxide (%) = [(A0 – A1) / A0] * 100, Where A0: absorbance of control (reaction mixture with extract vehicle only) and A1: absorbance of the sample extract. The concentration at which 50% of the reaction was completed (EC_{50}) was determined. The activities of the species were compared with ascorbic acid, which was used as positive control.

Estimation of Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay was used to determine the ability of the extracts to reduce ferric ions (Benzie and Strain, 1996). FRAP reagent was freshly prepared by mixing 2, 4, 6-Tripyridyl Triazine (TPTZ) and ferric chloride in sodium acetate buffer. The FRAP reagent was incubated at 37°C for 30 minutes. The final reaction mixture contained 25 µL of the extract (0.1-20 mg/mL) and 750 µL of FRAP reagent. The intensity of the blue/purple coloration was read at 593 nm (microplate reader: Bio Tek®, USA) against a blank of the buffer. A calibration curve of ferrous sulphate (0.2-2 mmol/mL) was used to express results in mmol Fe^{2+} per mL.

Preparation of extracts for enzymatic antioxidant activities

The enzymes were extracted using a modified protocol of Khan et al. (2015), whereby the 1 g of each macroalgae specimen was crushed in 50 mL of Tris-HCl, EDTA, $MgCl_2$ and PVP buffer and centrifuged at 15000g for 20 minutes at 4°C. Protein content was measured using the protocol of Bicinchoninic Acid (BCA) assay of Smith et al. 1985. To 10 µL of sample/Bovine Serum Albumin (BSA) (different concentration), 200 µL of a mixture of BCA and copper sulphate, followed by incubation at 37°C. The wavelength was read at 562 nm. The BSA curve was constructed, which was used to determine the amount of protein present in the sample.

Enzymatic antioxidant activities

Catalase (CAT) activity assay

The CAT activity was determined using the catalase activity colorimetric assay KIT according to manufacturer's instructions (Biovision, U.S.A). 30 µL samples and 1.5 µL positive control were respectively adjusted to 78 µL with assay buffer. Sample amount of samples and positive are placed in another well, followed by 10 µL stop solution to produce the High Control (HC). An H_2O_2 curve was plotted by measuring the optical density of a range concentration of H_2O_2 (0-10 nmol) at 570 nm (microplate reader: Bio Tek®, USA). Signal change by catalase for the sample i.e $\Delta A = A_{HC}-A_{sample}$. ΔA was applied to the H_2O_2 curve to obtain B, i.e. the nmol of H_2O_2 decomposed by catalase in 30 min reaction. The results were normalized to milligram of proteins used in the assay.

Glutathione Peroxidase (GPx) Activity

The GPx activity was determined using the GPx activity colorimetric assay KIT according to manufacturer's instructions (Biovision, U.S.A). To 30 µL of samples 30 µL of assay buffer was added. To 10 µL of positive control 40 µL of assay buffer was added. To each well the reaction mixture consisting of 33 µL of assay buffer, 40mM NADPH, 3 µL of glutathione reductase and 2 µL of glutathione synthase hydrogenase solutions were added. After fifteen minutes of incubation cumene hydroperoxide was added and was mixed thoroughly. The absorbance A_1 was read at 340nm at T1 and after 5 minutes to obtain A2. The GPx activity was obtained using the following equation: (A1-A2)-(RC_A1-TC_A2) to obtain B. Then, GPx activity = ((B)/(T2-T1)*V)*sample dilution. The

results were normalized to milligram of proteins used in the assay.

Superoxide Dismutase (SOD) assay

The SOD activity was determined using the superoxide dismutase activity colorimetric assay KIT according to manufacturer's instructions (Biovision, U.S.A). To 1 mL of WST solution, 19 mL of assay buffer was added and was mixed well. 15 µL of enzyme solution was then diluted with 2.5 mL of dilution buffer. Twenty (20) µL of sample was placed in well and separately in another well for blank 2, while 20 µL of water was placed in blank 1 and 3. Two hundred (200) µL of WST solution was added to each well. Twenty (20) µL of dilution buffer was added to blank 2 and blank 3. Twenty (20) µL of enzyme working solution was added to all samples and blank 1, and the mixture was incubated at 37°C for 20 minutes, following which the absorbance read at 450 nm (microplate reader: Bio Tek®, USA).

Data analysis

The normality tests were carried out using Shapiro-Wilk statistics. The means among different variables were compared using one-way ANOVA using SPSS 18.0. Tukey LSD as Post Hoc test was used to determine significant differences in mean antioxidant activities and phytochemicals among different species at different sites and during different experimental status.

RESULTS AND DISCUSSION

Variation of physico-chemical parameters and nutrient levels between HN and LN sites

Both at Day 0 and 7 the nutrients, including nitrate and phosphate levels differed among the two study site ($P<0.001$), being lower at the LN site compared to the HN site. Significant differences in photochemical parameters were recorded ($P< 0.001$), whereby pH was higher at HN sites and DO was higher at LN site (Figure 3 and Table 1).

Variation of phenolic contents and antioxidant activities

The non-enzymatic antixodiant activities and the phenolic contents varied significantly within and among the species at the different sites (Two-way ANOVA, $P<0.001$) at day 0 and 7 and before and after transplantation (Three way, $P <0.001$). The enzymatic anoxidant activities differed within and among the species at the two sites at day 0 (Catalase and SOD, Two-way ANOVA $P< 0.001$ and GPx, two-way ANOVA $P<0.05$), day 7 (Two-way ANOVA $P<0.001$) and before and after transplantation (Three-way ANOVA $P<0.01$). The photophysiological parameters differed significantly at the different sites, before and after transplantation (Two-way ANOVA $P<0.001$). No significant interspecific variation in photophysiological parameters was observed during the transplantation experiment (Table 2).

Variation of total phenolic contents and total flavonoid contents before and after transplantation experiment at HN and LN sites

The TPC was lower in all the species at the LN site compared to the HN site. Significant variation ($P < 0.001$) in TPC and TFC was observed among the different macroalgal species before and after transplantation to the LN and HN sites and vice versa. The TPC reduced significantly in all the species transplanted to the LN site and increased in all the species transplanted to the HN site. The TFC was significantly lower in *S. obovatum* at the LN site compared to that in the HN site. The TFC significantly increased in *S. obovatum* transplanted to HN site and decreased in those transplanted to the LN site (Figures 4A and B).

Variation of antioxidant efficacy pre and post-transplantation at sites with different nutrient levels

The DPPH and nitric acid scavenging activity and FRAP was stronger at HN site in all studied species compared to LN site. The DPPH scavenging activity and FRAP significantly increased in macroalgae transplanted to HN site and decreased in those transplanted to LN site.

The nitric oxide scavenging increased in *T. ornata* and *S. obovatum* transplanted to HN site and decreased in specimen transplant to LN site. The nitric oxide scavenging activity of *G. salicornia* increased in *G. salicornia* transplanted to HN site (Figures 5.A-C).

Variation of enzymatic antioxidant activity during transplantation experiment

Enzymatic antioxidant activities varied significantly in the macroalgae after seven days of transplantation (Table 2). The enzymatic antioxidant activities were lower in species at the LN sites compared to the HN site at day 0. At day 7, the GPx and the catalase activities and SOD activities significantly increased in all seaweed species transplanted to the HN site and decreased in all species transplanted to the LN site ($P < 0.01$) (Figures 6 A-C).

Variation of photophysiological parameters during transplantation experiment

Photophysiological parameters including quantum yield (F_v/F_m), ETR_{max}, NPQ_{max} and α (photosynthetic efficiency) and varied before and after transplantation between sites in the species (Figure 7). The quantum yield F_v/F_m increased in *S. obovatum* only after 7 days of transplantation at HN site. ETR_{max} and α were significantly higher in all species at HN site, both prior and after the experiment ($P<0.05$). In *S. obovatum*, NPQ_{max} significantly decreased when transplanted to LN site and increased in the speciemens transplanted to LN site. In *T. ornata*, NPQ_{max} rose after the transplantation in species transplanted to HN site, while in *G. salicornia*, no significant changes in NPQ_{max} were detected.

Correlations among the different parameters

During the transplantation experiment, at day 0, a moderate negative correlation was found between DPPH scavenging activity and pH. The Nitric oxide scavenging activity and total phenolic content positively correlated

with pH. At day 7, only the total phenolic content showed a moderate positive correlation between both nitrate and phosphate. Both at Day 0 and 7, the GPx, catalase and SOD strongly correlated with nitrate, phosphate, pH and DO (r >0.8). The maximum yield, α, ETR and NPQ correlated strongly (r > 0.8) with nitrate, phosphate, pH and DO (r >0.8).

Discussion

Different levels of nutrients, as well as physico-chemical parameters such as pH and DO were found at the two study sites. These may be attributed to dissimilar anthropogenic influences and different hydrodynamic processes at the two study sites. For example, the nitrate level was just 0.8 N mg/mL, the limit of nutrients in a recreational area (MoEHrDE 1999). Most human-induced nutrients enter coastal ecosystems from nearby agricultural sites, rivers and runoffs. Lower nitrate and phosphate levels were recorded at the LN site (Gris Gris) compared to the HN site (Bain Boeuf). The LN lagoon at the LN site is smaller and experiences stronger wave action, water flushing and water current compared to the HN site, which might help maintain lower nutrient levels at the LN site. The effect of some intertidal macroalgae was thus investigated under varied nutrient and physico-chemical conditions.

Table 1. Summary of one-way ANOVA test for variation in physico-chemical parameters and nutrients between HN and LN sites

Variables	Status		SS	DF	MS	F	P Value
Sites	Day 0	Nitrate	1.40	1	1.40	585.4	0.00
		Phosphate	0.01	1	0.01	124.6	0.00
		Salinity	0.10	1	0.10	1.0	0.34
		pH	0.99	1	0.99	738.2	0.00
		Temperature	0.13	1	0.13	2.0	0.18
		DO	1.36	1	1.36	440.5	0.00
	Day 7	Nitrate	1.40	1	1.40	585.4	0.00
		Phosphate	0.01	1	0.01	124.6	0.00
		Salinity	0.10	1	0.10	1.0	0.34
		pH	0.99	1	0.99	738.2	0.00
		Temperature	0.13	1	0.13	2.0	0.18
		DO	1.36	1	1.36	440.5	0.00

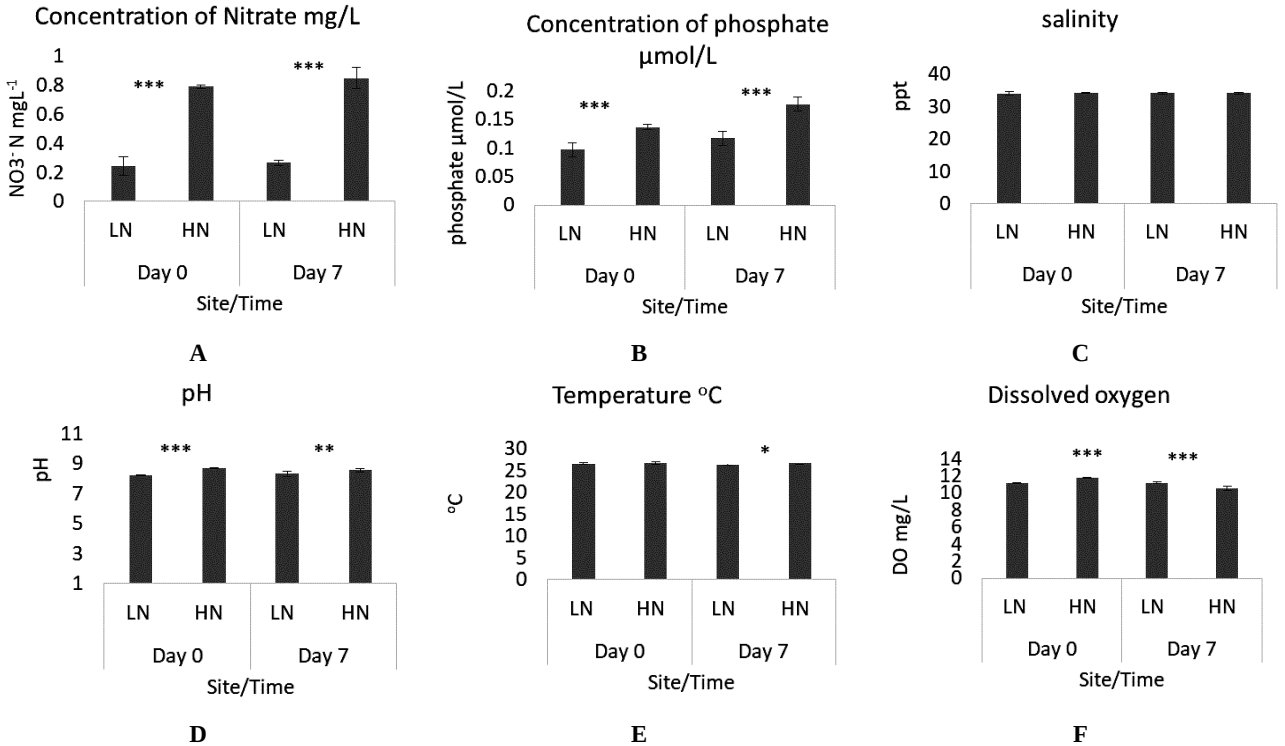

Figure 3. Nutrient levels (A and B) and (C-F) physicochemical parameters (n=3) varying at a different sites. The mean values ± SD are shown in the graphs. *** P < 0.001, **P < 0.01,* P < 0.05. (n=3)

Table 2. Variation of antioxidant activities, phytochemical contents among different species and photo physiological parameters during the transplantation experiment at the different sites. *** P< 0.001,**P < 0.01, *P<0.05 and the double and triple interaction of sites, transfer status and species

ANOVA	Status			SS	DF	MS	F	P value
Species * Site	Day 0	Non-enzymatic antioxidant activities	DPPH	18.8	2	9.4	105.4	***
			NO	338.5	2	169.3	578.5	***
			FRAP	0.1	2	0.1	81.9	***
		Phenolics	TPC	73.7	2	36.8	2443.6	***
			TFC	0.5	2	0.2	58.2	***
		Enzymatic antioxidant activities	GPX	0.4	2	0.2	4.8	*
			Catalase	0.1	2	0.1	46.5	***
			SOD	140.4	2	70.2	95.0	***
		Photophysiological parameters	Yield	0.0	2	0.0	3.8	-
			α	0.0	2	0.0	0.0	-
			ETRmax	43.2	2	21.6	1.6	-
			NPQmax	0.0	2	0.0	0.1	-
	Day 7	Non-enzymatic antioxidant activities	DPPH	15.3	2	7.7	119.6	***
			NO	169.1	2	84.5	2534.9	***
			FRAP	0.1	2	0.0	80.2	***
		Phenolics	TPC	132.0	2	66.0	2797.8	***
			TFC	0.7	2	0.3	1053.3	***
		Enzymatic antioxidant activities	GPX	3.2	2	1.6	17.7	***
			Catalase	0.0	2	0.0	42.9	***
			SOD	167.7	2	83.9	203.9	***
		Photophysiological parameters	Yield	0.0	2	0.0	5.2	*
			α	0.0	2	0.0	12.2	**
			ETRmax	4.4	2	2.2	0.0	-
			NPQmax	0.0	2	0.0	2.1	-
Site Status		Non-enzymatic antioxidant activities	DPPH	28.5	1	28.5	372.3	***
			NO	30.8	1	30.8	177.2	***
			FRAP	0.1	1	0.1	140.6	***
		Phenolics	TPC	60.4	1	60.4	3122.7	***
			TFC	0.0	1	0.0	1.2	-
		Enzymatic antioxidant activities	GPX	459.4	1	459.4	6645.2	***
			Catalase	5.0	1	5.0	5582.7	***
			SOD	591.1	1	591.1	1028.1	***
		Photophysiological parameters	Yield	0.0	1	0.0	0.5	-
			α	1.6	1	1.6	275.4	***
			ETRmax	11346.9	1	11346.9	234.2	***
			NPQmax	4.0	1	4.0	742.2	***
Species * Site * Status		Non-enzymatic antioxidant activities	DPPH	33.4	2	16.7	218.2	***
			NO	483.6	2	241.8	1483.6	***
			FRAP	0.1	2	0.1	103.9	***
		Phenolics	TPC	201.2	2	100.6	5204.5	***
			TFC	0.0	2	0.0	2.6	-
		Enzymatic antioxidant activities	GPX	3.0	2	1.5	21.9	***
			Catalase	0.1	2	0.1	67.3	***
			SOD	10.9	2	5.5	9.5	**
		Photophysiological parameters	Yield	0.0	2	0.0	0.7	-
			α	0.0	2	0.0	2.0	-
			ETRmax	10.8	2	5.4	0.1	-
			NPQmax	0.0	2	0.0	0.1	-

The phenolic contents, antioxidant efficacy, enzymatic antioxidant activities and photophysiological factors varied significantly between Bain Boeuf, characterized by Higher Nutrient level (HN site) and Gris Gris, characterized by Lower Nutrient level (LN site). The phenolic contents and in vitro antioxidant efficacy were higher in most studied macroalgal species at HN site in comparison to LN site. Antioxidant efficacies like ferric reducing antioxidant potential correlated with TPC and TFC. The transplantation of species from HN to LN sites resulted in an increase in non-enzymatic antioxidant activities and phenolic levels, while the reverse trend was observed when the species

were transferred from HN to LN sites. DPPH scavenging activity correlated positively with nitrate, phosphate, pH and DO, indicating antioxidant response of macroalgal species to changing amount of nutrients and physicochemical parameters. The presence of phenolic compounds with antioxidant properties usually increase under stressful condition (Salar et al. 2013). While, increased antioxidant activities and levels of reactive oxygen scavenging enzymes are known to occur as a stress response in macroalgal species (Collen and Davison 1999). Non-enzymatic The conditions at the LN site thus seems to be considerably low, reducing the phenolic level and non-enzymatic antioxidant activities compared to site with higher nutrient occurrence.

Enzymatic antioxidant activities varied significantly during the transplantation experiment. Enzymatic antioxidant enzyme such as Superoxide Dismutase (SOD) are known to act as the first line of defence against oxidative stress by converting oxygen radicals to oxygen and hydrogen peroxide (Alscher et al. 2002), while catalase (CAT) (Bhaduri and Fulekar, 2012) and glutatione peroxidase (Gaetani et al. 1989) catalyse the convertion of hydrogen peroxide into oxygen and water. Levels of intracellular enzymatic antioxidant activities increase under stressful conditions for e.g. CAT and Ascorbate peroxidase (APX) activities increased under nitrogen depleted conditions in the algae *Dunaliella salina* (Yilancioglu et al. 2014). In the current study, the enzymatic activities were initially distinct at the two sites, being higher in HN site and lower in the LN site. Most of the enzymatic antioxidant activities decreased in species transplanted to LN site and increased in species transplanted to HN site. The SOD activity only altered significantly in *S. obovatum* transplanted to HN site, while no significant changes were observed in the SOD activity of *G. salicornia* and *T. ornata*. The GPx and the catalase activities increased in all macroalgal species significantly after transplantation to the HN site and decreased considerably after transplantation to the LN site. Enzymatic antioxidant (GPx and catalase) activities correlated positively with the nitrate, phosphate and dissolved oxygen level, indicating that lower nutrient levels, pH and higher dissolved oxygen level at LN site may have contributed in the lower expression of enzymatic antioxidant activities and phenolic contents in macroalgae. A possible mechanism of increase in antioxidant activities that may occur, as an increase in nutrient levels in marine system is known to entail the limitation of other nutrients, following which levels of antioxidant increase (Turner et al. 2003). At the LN site a balance in all nutrient levels may have thus inhibited a stress state.

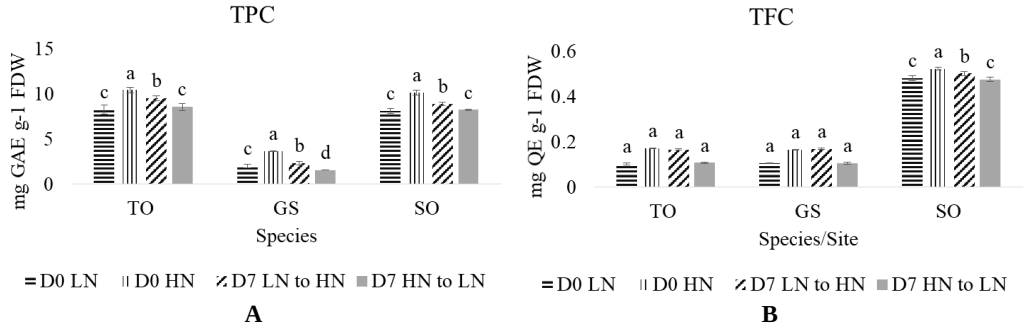

Figure 4. Variation of TPC (A) and TFC (B) in species before transplantation (D0) and seven days after transplantation (D7) to sites with different nutrients level. TO-*T. ornata*, GS-*G. salicorna* and SO-*S. obovatum*. The alphabets indicates differences in a species at a different site and before and after transplantations. (n = 3)

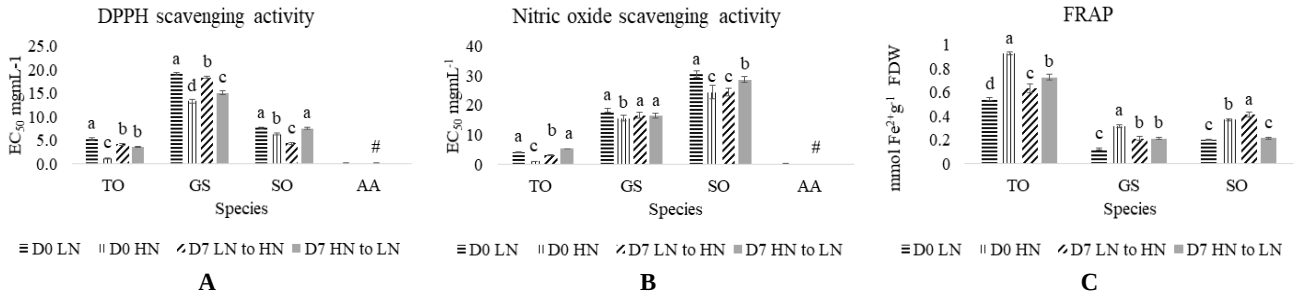

Figure 5. Variation of antioxidant efficacies [DPPH scavenging activity (A), nitric oxide scavenging activity (B) and FRAP (C)]) in species before transplantation (D0) and seven days after transplantation (D7) to sites with different nutrients level. TO-*T. ornata*, GS-*G. salicorna* and SO-*S. obovatum*. LN-Lower Nutrient, HN-Higher nutrient. The alphabets indicates differences in a species at a different site and before and after transplantations. # indicates the difference between the standard and the seaweed species (n = 3)

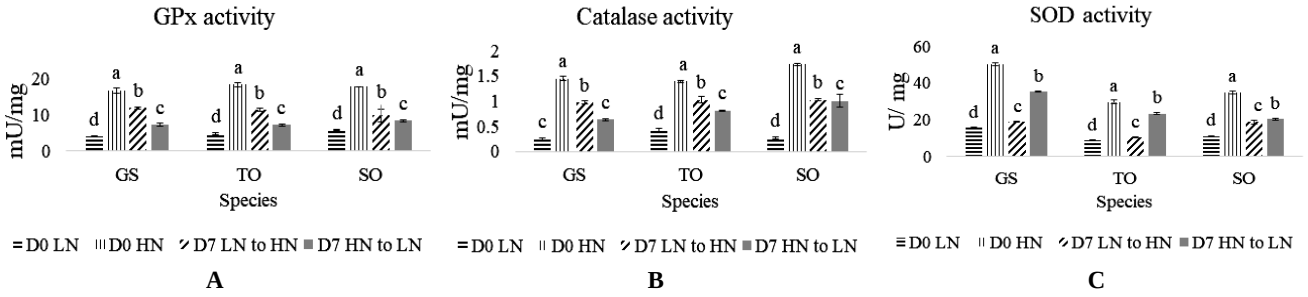

Figure 6. Variation of the enzymatic antioxidant activities, including GPx (A), Catalase (B) and SOD activities (C) in species before transplantation (D0) and seven days after transplantation (D7) to sites with different nutrients level. TO-*T. ornata*, GS-*G. salicorna* and SO-*S. obovatum*. LN-Lower Nutrient, HN-Higher nutrient. The alphabets indicates differences in a species at a different site and before and after transplantations

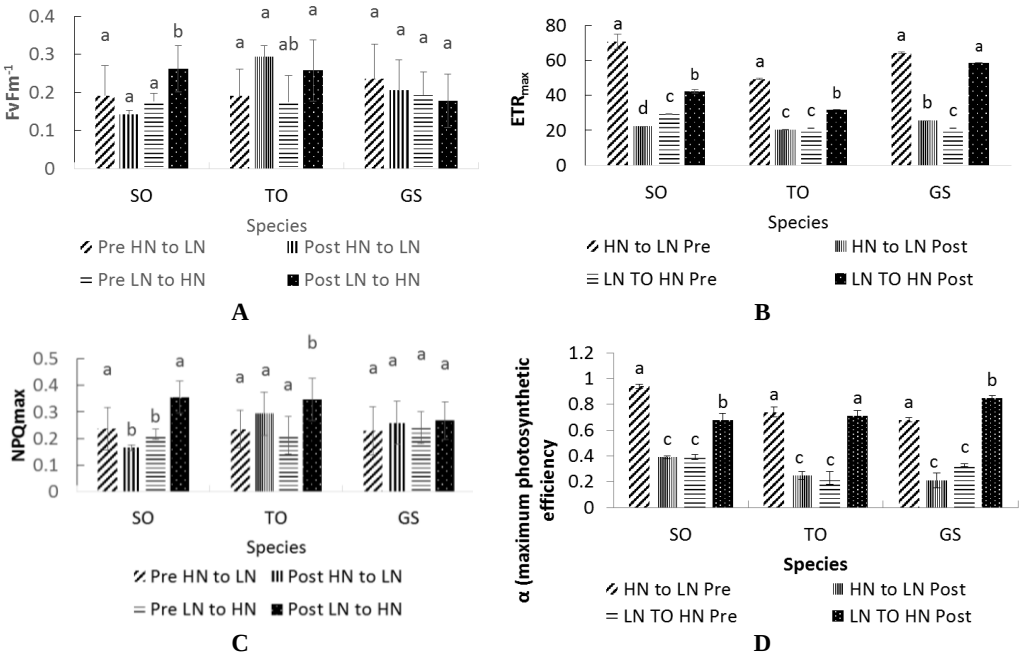

Figure 7. Variation of photophysiological parameters, including Yield (quantum yield) (A) ETR_{max} (B), NPQ_{max} (C) and α (photosynthesic efficiency) (D), in species before transplantation (D0) and seven days after transplantation (D7) to sites with different nutrients level. TO-*T. ornata*, GS-*G. salicorna* and SO-*S. obovatum*. LN-Lower Nutrient, HN-Higher nutrient. The alphabets indicates differences in a species at a different site and before and after transplantations

The highest quantum yield (Fv/Fm) indicates photoinhibition (as the balance of photodamage and repair) and Electron Transport Rate (ETR) photosynthetic capacity (Schreiber et al. 1995). ETRmax and α were significantly higher in all species at HN site, both in resident and transplanted specimens. The quantum yield Fv/Fm increased in *S. obovatum* only after 7 days of transplantation at HN site, while it was similar in the other species before and during other transplantation. This indicates that species like *T. ornata* and *G. salicornia* had better adaptability to photo-physiological stress. The *H. opuntia*'s photosynthetic capacity, efficiency, and photo-protection increased under increased nutrient levels (Teichberg et al. 2013). The maximum yield α, ETR and NPQ correlated strongly and positively with nitrate, phosphate, pH and DO, indicating the possible role of nitrate as well as phosphate, pH and DO in the differences observed. The net photosynthesis rate and Fv/Fm of nitrate-treated Sorghum plants under Cd stress were higher than that of ammonium-treated plants when the pH was unbuffered (Bai et al. 2021). The antioxidant level in watermelon fruits increased three times in plats exposed to 30 mgL^{-1} silver nitrate concentration (Cabrera-De La Fuente et al. 2014). This may suggest the possible investigation of the role of nitrate level in enhancing pH and its effects on photosynthetic efficiency.

The photosynthetic electron transport through PSII under excessive light is limited through non-photochemical quenching (NPQ) (Muller et al. 2001; Schagerl and Möstl 2011). In *S. obovatum*, NPQmax significantly decreased when transplanted to LN site, indicating less photoinhibition, probably due to lesser stress at the site. In

T. ornata, NPQmax rose after the transplantation in species transplanted to HN site, indicating increased photoinhibition due to selective pressure at the site. While in *G. salicornia*, no significant change in NPQmax was detected, possibly indicating its adaptation to growing under varied conditions and suggesting low damage to the photosynthetic reaction center at PSII. ROS production is associated with PAR-driven electron transport (Rijstenbil et al. 2000). ROS production is linked with impairment of the xanthophyll cycle, followed by non-photochemical quenching in samples exposed to the full solar range (Rijstenbil et al. 2000; Krause and Jahns 2004; Asada 2006). In our case, it can be implied that light stress contributes to ROS production in *S. obovatum*. NPQ was not impaired, especially in *G. salicornia* and *T. ornata*, reflecting a successful response to macroalgae stress, possibly by producing antioxidants, preventing damages, e.g., photooxidation of tissues the pigments. These results indicate that macroalgae living under higher nutrient levels have greater adaptation, including that to light stress and are able to dissipate energy produced during light stress. These findings indicate that increased nutrient levels may be linked to increased oxidative stress, followed by enhanced production of photo-protective pigments and secondary metabolites to better cope with oxidative stress. Nevertheless, further studies on oxidative stress are needed, as inferring oxidative stress using antioxidants only can be erroneous (Costantini and Verhulst 2009).

In conclusion, the different sites had distinct environmental conditions that led to their characterization as LN site, with lower nutrients, pH and higher dissolved oxygen levels and HN site, with comparatively higher nutrients, pH and lower dissolved oxygen levels. The phenolic contents and antioxidant activities of macroalgae may be influenced by varied nutrient levels and physico-chemical parameters existing in its habitat. Enzymatic antioxidant activities like glutathione peroxidase and catalase were more sensitive at reflecting the lower oxidative stress and oxidative stress acclimatisation occurring at the HN site, followed by non-enzymatic antioxidant activities and phenolic contents. ETRmax and α were significantly higher in all species at HN site, both in resident and transplanted specimens. The quantum yield Fv/Fm increased in *S. obovatum* only after 7 days of transplantation at HN site, while it was similar in the other species before and during other transplantation. This indicates that species like *T. ornata* and *G. salicornia* had better adaptability to photophysiological stress. In *S. obovatum*, NPQmax significantly decreased when transplanted to LN site, indicating less photoinhibition, probably due to lesser stress at the site. In *T. ornata*, NPQmax rose after the transplantation in species transplanted to HN site, indicating increased photoinhibition due to selective pressure at the site. While, in *G. salicornia*, no significant change in NPQmax was detected, possibly indicating its adaptation to growing under varied conditions and suggesting low damage to the photosynthetic reaction center at PSIILower nutrient levels may thus be linked to reduced oxidative stress, which is followed by the lesser need of photo-protection and secondary metabolites. Macroalgal species may thus play an essential role as a natural indicator of the nutrient levels in of lagoon system.

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The red coralline alga *Hydrolithon onkodes*, an attractor of coral larvae, is photosynthetically more susceptible to thermal stress than *Lithophyllum incrustans*

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Abstract. *Perrine MJS, Mundil S, Kaullysing D, Bhagooli R. 2023. The red coralline alga* Hydrolithon onkodes*, an attractor of coral larvae, is photosynthetically more susceptible to thermal stress than* Lithophyllum incrustans*. Indo Pac J Ocean Life 7: 91-99.* Red Coralline Algae (RCAs) are important components of coral reefs and are involved in reef-building via calcification, cementation, the synthesis of anti-fouling compounds and of chemicals to aid recruitment, settlement and metamorphosis of reef species. This study aimed to investigate the distribution of RCAs at four sites around Mauritius Island and the effects of thermal stress on the effective photosynthetic yield of photosystem II (Φ_{PSII}) of two species of RCA namely, *Lithophyllum incrustans* (Philippi, 1837) and *Hydrolithon onkodes*, known to attract coral larvae. Out of the nine RCA species observed, two non-geniculate RCAs, *H. onkodes* and *L. incrustans*, were among the most dominant, especially at the lagoonal and reef zones of the four studied sites Flic en Flac, Belle Mare, Trou aux Biches and Flat Island. These two RCAs were collected, acclimated for 24 hours on a 12h:12h dark-light cycle and then exposed to 27°C, 30°C and 33°C for 3 (T_3), 6 (T_6) and 19 (T_{19}) hours. After 3, 6 and 19 hours, relative change in Φ_{PSII} compared to initial (T_0) was used for comparison among tested species. At 27°C the Φ_{PSII} did not fluctuate significantly during the experiment for both RCAs. At a temperature 30°C only *H. onkodes* significantly decreased from 0.541 ± 0.54 at T_0 to 0.445 ± 0.116 at T_{19}. At 33°C, *L. incrustans* showed a significant decline of 24.82 ± 7.4% while *H. onkodes* decreased by 90 ± 12.6%. Visual observations revealed that *H. onkodes* changed from the initial healthy-looking color of light grey to pale purple after thermal exposure. These findings indicate that the coral larvae-attracting *H. onkodes* is more susceptible than the *L. incrustans* to thermal stress, implying subsequent possible impacts on coral recruitment process especially in the wake of climate change-driven ocean warming.

Keywords: PAM fluorometry, photosynthetic parameters, red coralline algae, thermal tolerance

INTRODUCTION

Worldwide mean surface temperature has so far increased by almost 0.87°C in the last one and a half centuries (during the interval 1850-2015) as reported by International Panel on Climate Change (IPCC) and will probably rise more by 3°C by the end of this century (Masso-Delmotte et al. 2018). Rising ocean surface temperature is amongst the chief impacts disturbing marine ecosystems (Stenseth et al. 2002), which can affect the abundance and distribution of marine organisms, and likewise lead the way to extinction of populations situated at the extremity of their thermal tolerance (Sanford et al. 2019). Moreover, a marine heatwave can intensely impact ecosystem function and structure by provoking extensive community, change species range shift and mortality (Jentsch et al. 2007).

Red Coralline Algae (RCAs) are classified in the division Rhodophyta and form part of a distinctive order Corallinales. The deposition of calcium carbonate around and inside the algal thalli and the presence of calcium carbonate in the wall give the thalli a hard and rigid construction which is the main feature of the coralline algae (Richmond 1997). Globally spread, RCAs are considered major components of coastal ecosystem structure and function (Basso 1998). However, few studies have been conducted around Mauritius and its adjacent islands and waters on RCA distribution with species such as *Hydrolithon* sp. and *Renouxia* sp. (Ballesteros and Afonso-Carrillo 1995; de Clerck et al. 2004).

The ecological importance of RCAs varies from playing an essential function as ecosystem engineers of greatly heterogeneous communities (Foster 2001) to being the favored surface for settlement for invertebrate larvae (Williams et al. 2008). Key ecological processes affecting the distribution pattern and abundance of associated species are widely recognized as biotic interactions involving RCAs (Seabra et al. 2019). This interaction can derive from the structure and persistence of communities within a benthic ecosystem (Nelson 2009). Globally, several studies have characterized the significant role of RCAs in the marine ecosystem, for instance, foundation species (Steneck and Dethier 1994), reef frameworks (Adey 1978; Richards and O'Leary 2015), coral larval preference for

RCAs (Elmer et al. 2018) and community structure (Kennedy et al. 2017; Lei et al. 2018; Schoenrock et al. 2018). RCAs strengthen the skeletal structure of the non-living coral and seal fissure in the reef substratum, thus sustaining topographic complexity and decreasing reef erosion (Fabricius and De'ath 2001). RCA can be primary reef builders that provide a substrate for settlement for additional organisms (Elmer et al. 2018). RCAs are characteristically believed to improve recruitment or trigger metamorphosis of larvae of further species through contributing biochemical cues (O'Leary et al. 2012) or by offering enough structural heterogeneity. These strategies are vital for the invertebrate communities' diversity in the tropic and temperate regions (McCoy and Kamenos 2015).

However, with an increase in temperature, calcification and growth rate of RCAs rise within the array of normal environments (Basso 2012) and above these optimal thermal levels, heating is harmful and causes narcosis and death. Some studies have explored the effect of temperature on RCA where one study carried out showed that after being kept at 32°C for 7 days, RCAs clearly showed signs of bleaching and the makeup of biofilm on the surface of the RCAs had significantly changed (Webster et al. 2011). Despite various studies demonstrating that RCAs are sensitive to rise in temperature, no study has defined the upper, lower and optimal photosynthetic temperature of tropical RCAs. Moreover, no study has documented the effect of steadily increasing temperature on non-geniculate RCAs, under current and predicted climate change scenarios. It is noteworthy that limited studies on RCAs have been conducted around Mauritius in the Western Indian Ocean, though studies on other macroalgae of the Republic of Mauritius have been documented (Bolton et al. 2012; Somanah et al. 2012; Mattio et al. 2013; Ramah et al. 2014, 2021a,b; Kaullysing et al. 2016; Bhagooli and Kaullysing 2019; Gopeechund et al. 2020; Bhagooli et al. 2021a,c,d; Narrain et al. 2023). To explore the effect of temperature on the photo-physiology of non-geniculate RCAs, two species were used to assess the photo-physiological thermal tolerance in laboratory-based experiments.

Despite the importance of RCAs, their sensitivity to increasing temperature is unclear (Martin et al. 2013). Therefore, it is worth understanding the biological response of climate-sensitive organisms to short-term change (Jentsch et al. 2007). The study's objectives were to expose the two fresh acclimated species of RCA to short temperature stress to test their tolerance and to use a Pulse Amplitude Modulated (PAM) fluorometer to determine the effective photosynthetic yield of photosystem II (Φ_{PSII}). The temperature was ramped up from 27°C to 33°C to get an insight into its impact on the photosynthetic activity due to heat stress.

MATERIALS AND METHODS

Distribution of RCAs and corals

The study was carried out at three zones, namely, near-coast, lagoon and reef at four sites, Belle Mare (BM), Trou aux Biches (TB), Flic en Flac (FEF) and Flat Island (FI) around Mauritius (Figure 1). The water depth ranged from 1-1.7 m at low tide. Three transects each of 20 m length were laid parallel to the reef at the near-coast, lagoon and reef zones. Quadrats of 50 cm x 50 cm were placed at random intervals on the left and right of the line transect of 20 m and RCAs and corals were recorded using video transect method (Leujak and Ormond 2017). Video recording at a speed of 7 m/minutes at an angle of 45° and in situ photos of the RCAs were captured using a digital Olympus TG5 camera by snorkeling. The percentage of RCA cover, dead and live coral cover were determined through the video survey.

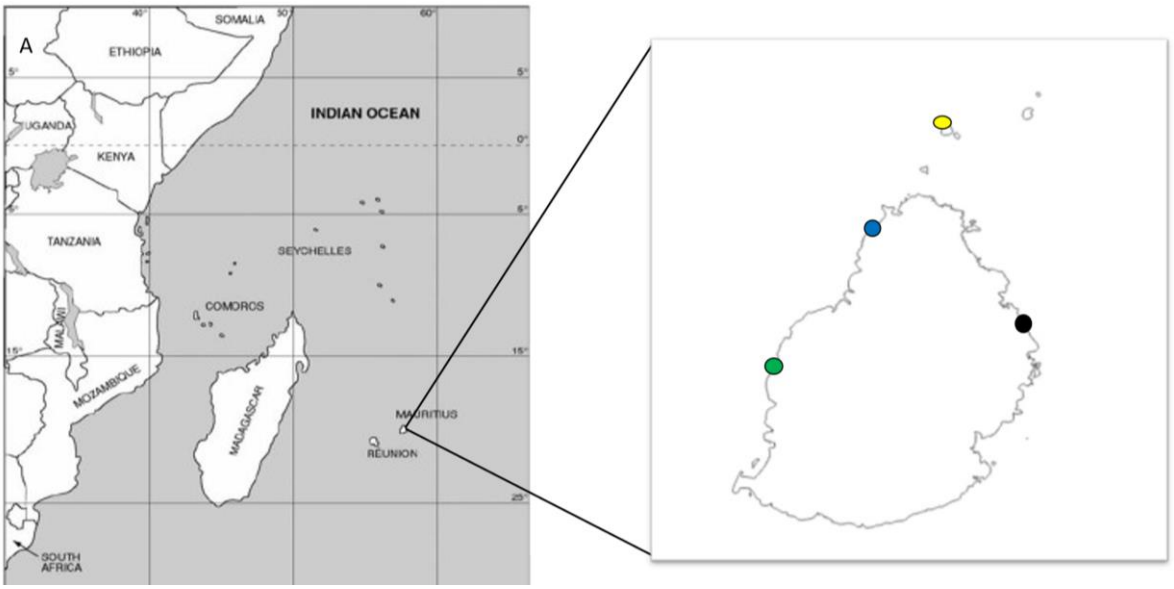


Figure 1. A. map of Indian Ocean showing location of Mauritius. B. Map showing studied areas: Belle Mare (*black*), Trou aux Biches (*blue*), Flic en Flac (*green*), and Flat Island (*yellow*)

Field surveys were carried out in October and November 2019, and January and February 2020. The lagoon and reef zones are rather flat, with similar exposure to light and RCA samples were harvested in-depth ~ 1.2 m in lagoon and ~ 1 m in reef zone using hammer and chisel and/or handpicked from the four sites while snorkeling. The samples were preserved in seawater from the station during transportated to the laboratory. RCA samples were preserved at-20°C for subsequent identification under a light microscope according to Woelkerling et al. (1993).

Sampling of RCAs for thermal experimentation

Fragments from two species of RCAs, namely, *Lithophyllum incrustans* (Philippi, 1837) and *Hydrolithon onkodes* ((Heydrich) D.Penrose & Woelkerling), known to attract coral larvae, were collected on the same day during early hours using a blade at depths not exceeding 1.5 m at BM. Following collection, the two species of RCAs were inserted in a clear plastic bag with seawater and were maintained in ambient collection conditions by placing them in a covered container filled with seawater while transportation to the laboratory. The two RCAs were transferred to aquaria in the laboratory within one hour of collection and were acclimated for 24 hours with continuous air supply.

Laboratory thermal stress experiment

The effect of temperature on photosynthetic yield of photosystem II (Φ_{PSII}) of the two RCAs were experimentally investigated. Nine aquaria were used and were arranged in a set of three rows labelled with temperature 27°C (control), 30°C and 33°C. Both species were placed in all aquaria containing seawater at a salinity 34 ppm and the temperatures were ramped up to 30°C and 33°C within 3 hours. The temperature of each aquarium was controlled constantly using thermostats 50W aquarium heater (Eco-therm, aquarium system). The temperature was checked regularly and monitored using a hand-held thermometer. Each aquarium was aerated to maintain a constant oxygen and a homogeneous temperature condition. The control was set at room temperature (~27°C) and the treatments were set at 30°C and 33°C, and after that a constant temperature was maintained in the respective aquaria.

Chlorophyll-*a* fluorescence measurement

The effective quantum yield at photosystem II (Φ_{PSII}) was determined using a Diving-Pulse-Amplitude-Modulated (PAM) fluorometer (Bhagooli et al. 2021b). A saturating pulse of 4000 µmol quanta m^{-2} s^{-1} was applied by placing a 5 mm fiber optic probe on the thallus and a weak light emission of <1 µmol quanta m^{-2} s^{-1} was used to measure the maximum (F_m) and minimum (F_t) fluorescence, respectively. The Φ_{PSII} was then calculated as follows (Genty et al. 1989):

$$\Phi_{PSII} = (F_m-F_t)/F_m = \Delta F/ F_m$$

Initial observations were made and readings taken (T_0) prior to the RCAs being placed in the respective treatments. Further readings were taken after 3 (T_3), 6 (T_6) and 19 (T_{19}) hours after exposure to the thermal conditions.

Statistical analysis

Statistical analysis was conducted in SPSS. First, all data were tested for normality using the Shapiro-Wilk test (data was normally distributed P value>0.05). One-way ANOVA and Post Hoc Tests were performed to determine whether zones influenced growth form distribution at each site studied. One-way ANOVA was also carried out to investigate the effect of temperature and exposure period on the effective quantum yield of the two species of RCA. Finally, Principal Component Analysis (PCA) was performed on the data related to species distribution of RCA found occurring at the study sites in Mauritius to investigate their correlation.

RESULTS AND DISCUSSION

Distribution of substrate, corals, and RCAs around Mauritius

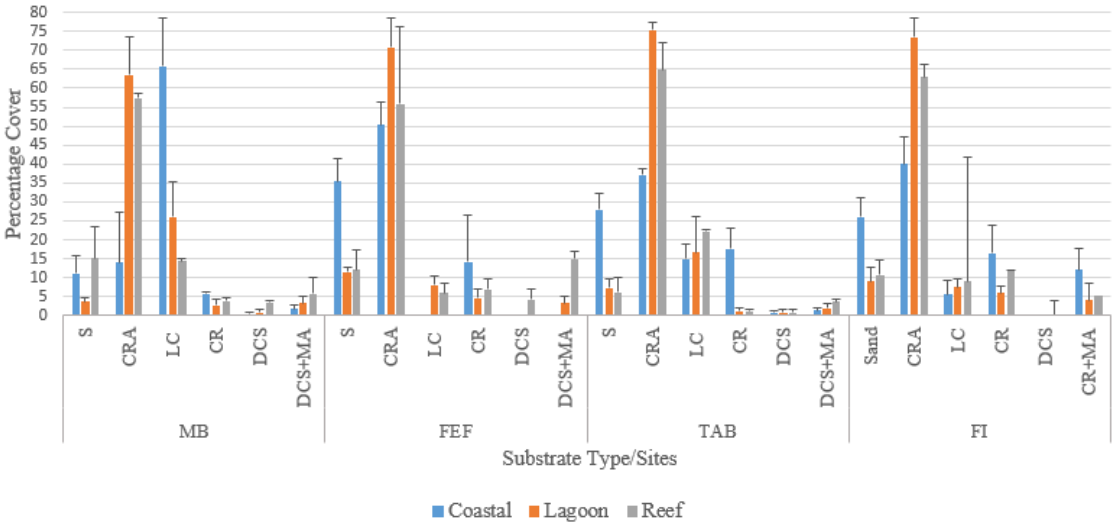
The coastal substrate was dominated by live coral (65.9±4.55%) at BM and coral rubble was at 5.00±0.29% (Figure 2A). The same tendency was found in the lagoon with 26.0±1.45% live coral and 1.40±0.8% coral rubble. On the reef, sand dominated by 15.2±1.64% and dead coral stand was the least at 3.4±0.74%. At FEF, the dominant substrate at the coastal zone was sand at 35.5±6.09% and the coral rubble was the lowest at 14.1±1.31%. Sand was dominant in the lagoon with 11.5±6.09% and dead coral stand with macroalgae (DCS+MA) the least at 3.20±1.88%. On the reef there was a dominance of DCS+MA with 14.9±3.32% and DCS was lower at 4.20±2.72%. Coastal substrate at TB was dominated by sand at 28.0±4.31% and DCS+MA was least at 1.40±0.73%. Live coral had the highest percentage cover of 16.7±9.53% in the lagoon and DCS was the lowest at 0.90±0.62%. Data indicated a dominance of live coral cover at the reef with 22.2±6.69% and DCS was the lowest at 0.80±0.40%. Flat Island coastal zone was dominated by sand with a percentage cover of 26.0±5.25% and live coral the lowest at 5.6±3.70%. In the lagoon, sand had the highest percentage cover with 9.0±3.82% and DCS+MA was at 4.37±4.0%. The reef substrate was dominated by sand at 10.7±1.91% and DCS+MA was at 5.4±0.78%.

PCA results indicated that *L. kotschyanum*, *L. incrustans* and *Phymatolithon purpureum* were most dominant at TB (Figure 2B, Table 1). The *H. gardineri* was most dominant at FEF but least dominant at BM. The *H. onkodes* was most dominant at FI. The RCAs were higher in the lagoon and reef sites compared to the coastal zones at all the four study sites (Figure 3A). The RCAs varied in composition among the sites with a single species occurring at the coast of BM and six species at the lagoon of FEF and TA out of the total of 11 species of RCAs observed around Mauritius (Figure 3B). Only one species of non-geniculate RCA, *H. onkodes* was found growing in near-coast at BM. Four Species were recorded in the lagoon, namely, *H. gardineri*, *H. onkodes*, *L. incrustans*

and *P. purpureum*, and six species were recorded on the reef: *H. gardineri*, *H. onkodes*, *L. incrustans*, *L. kotschyanum*, *L. cabiochae* and *P. purpureum*. From the five genera, seven species of RCAs were recorded at Flic en Flac. Four species were identified in the near-coast zone: *P. purpureum*, *L. incrustans*, *H. onkodes* and *H. gardineri*. Five species, namely, *H. gardineri*, *H. onkodes*, *L. incrustans*, *L. kotschyanum* and *Phymatolithon* were found occurring in the lagoon and reef zones. At TA, four species, *H. gardineri*, *H. onkodes*, *L. incrustans* and *P. purpureum*, were recorded at the near-coast zone. Six species were observed in both lagoon and reef zones at TB. Six species were observed at the reef zone at FI: *H. gardineri*, *H. onkodes*, *L. incrustans*, *L. kotschyanum*, *P. purpureum*, and *L. laevigatum*.

Table 1. PC loading for the PCA

	RCA species				
Sites	*H. gardineri*	*H. onkodes*	*L. incrustans*	*L. kotschyanum*	*P. purpureum*
BM_C	0.00	100.00	0.00	0.00	0.00
BM_L	6.90	65.52	17.24	0.00	6.90
BM_R	17.78	33.33	24.44	2.22	6.67
FEF_C	49.15	11.86	22.03	0.00	16.95
FEF_L	25.71	17.14	28.57	5.71	17.14
FEF-R	26.32	18.42	26.32	5.26	21.05
TB_C	7.55	24.53	41.51	0.00	26.42
TB_L	10.87	19.57	28.26	8.70	23.91
TB_R	8.62	20.69	39.66	5.17	15.52
FI_C	7.14	42.86	21.43	3.57	25.00
FI_L	6.02	45.78	26.51	2.41	19.28
FI_R	6.45	41.94	33.87	1.61	12.90



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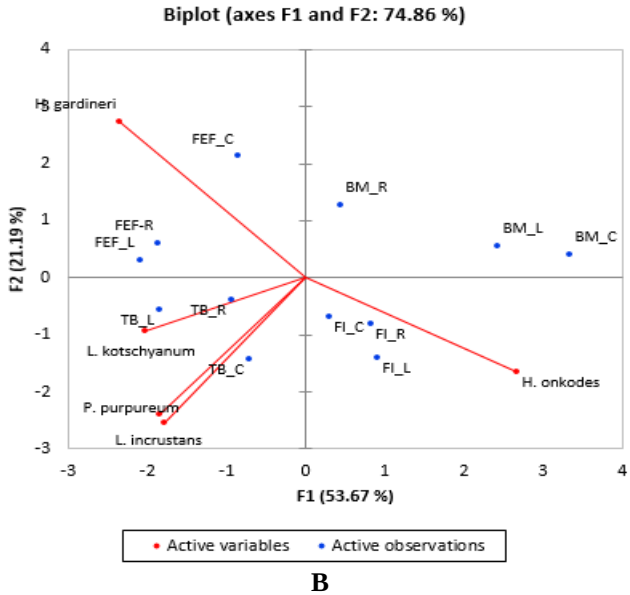

B

Figure 2. A. Distribution of substrate and biota at Belle Mare (BM), Flic en Flac (FEF), Trou aux Biches (TAB), and Flat Island (FI) around Mauritius. Coralline red algae (CRA), live coral (LC), Coral rubble (CR), Dead coral stand (DCS) and dead coral stand with macroalgae (DCS+MA). B. Principal Component Analysis (PCA) for RCAs for four sites around Mauritius Island

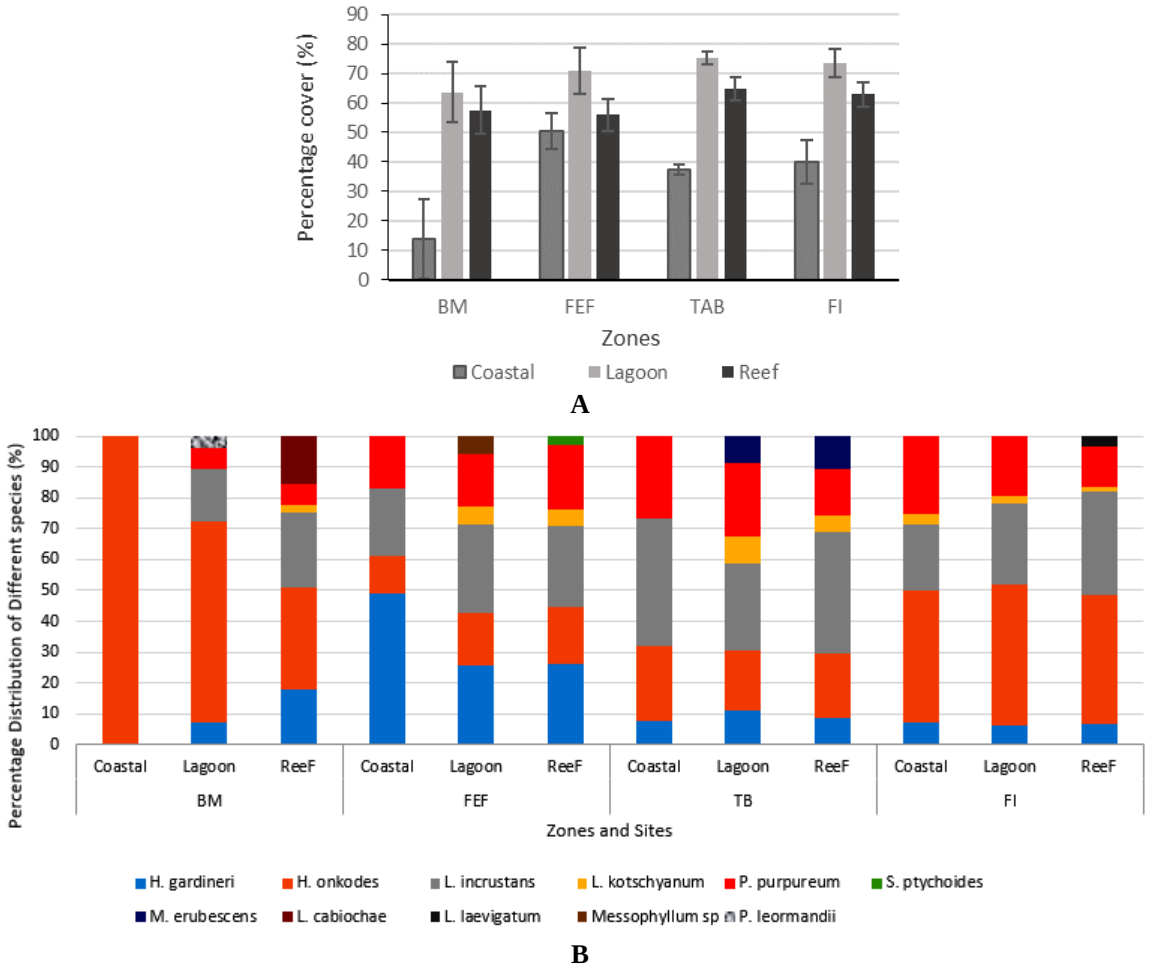

Figure 3. A. Distribution of Red Coralline Algae (RCAs) in terms of percentage cover (Mean ± SD, n=3) and B. RCA species at the four studied sites around Mauritius Island

Chlorophyll-*a* fluorescence thermal responses of *H. onkodes* and *L. incrustans*

The effective quantum yield at PSII (Φ_{PSII}) decreased significantly after 6 hours (P=0.0027) and 19 hours (P=0.002) at both 30°C and 33°C treatments in *H. onkodes* when compared to 27°C treatment, which remained unchanged during this exposure period (P>0.05) (Figure 4A). While in *L. incrustans*, Φ_{PSII} decreased significantly only at 33°C after 19 hours (P= 0.044) of treatment (Figure 4B). Relative changes indicated insignificant changes in 27°C treatment (P>0.05) for both RCAs throughout the experimental period (Figure 5A). At 30°C, only Φ_{PSII} in *H. onkodes* decreased by approximately 20% (Figure 5B), while at 33°C after 19 hours, Φ_{PSII} decreased by 20% and 98% in *L. incrustans* and *H. onkodes*, respectively (Figure 5C).

Visual observations of thermally treated RCAs

Both RCAs at 27°C showed no signs of visual bleaching and the healthy color was maintained throughout the experiment. At 30°C, *H. onkodes* exhibited a change in color from grey-white to an uneven pale-purple across all specimens in the aquaria after approximately 10 hours of exposure, whereas after only six hours of continuous exposure to 33°C, a change in color was observed in all specimens of *H. onkodes*. After 19 hours of exposure, specimens of *H. onkodes* in both 30°C and 33°C had visible signs of visual bleaching where a pale-purple color was observed in all specimens (Figure 6). On the other hand, *L. incrustans* showed a slight change in color at 33°C after 19 hours of exposure and no apparent changes in specimens exposed to 27°C and 30°C conditions (Figure 7).

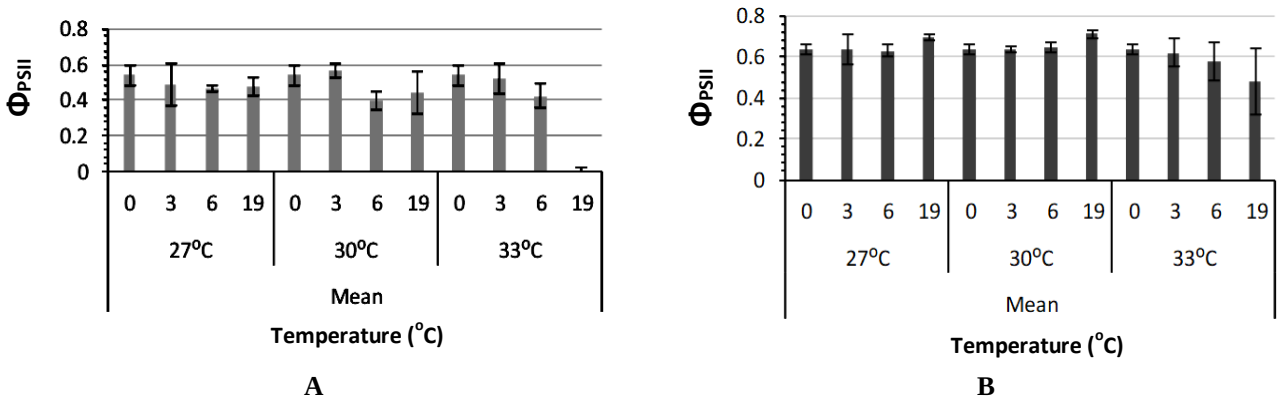

Figure 4. Effective quantum yield at PSII (Φ_{PSII}) in *Hydrolithon onkodes* (A) and *Lithophyllum incrustans* (B) exposed at 27°C, 30°C and 33°C for a period of 19 hours

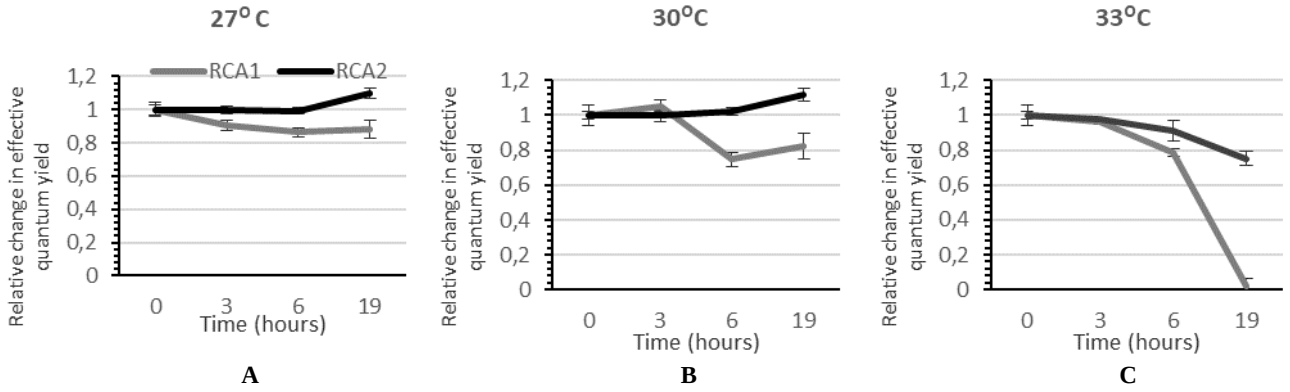

Figure 5. Relative (to initial) changes in Φ_{PSII} in *H. onkodes* (RCA1) and *L. incrustans* (RCA2) at 27°C, 30°C and 33°C exposures (Mean ±SD, n=3)

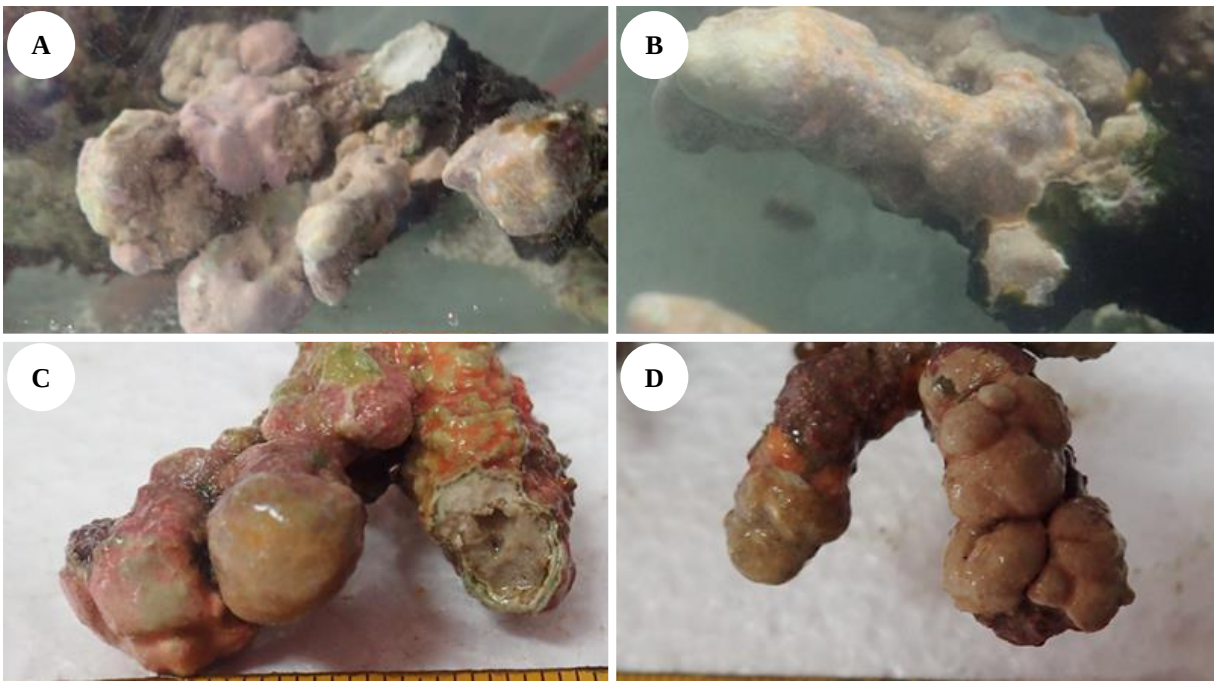

Figure 6. Visual observation of colors before and after thermal experimental trials on RCA *H. onkodes*. Color observed: (A) Healthy color before experiment, color following 19 hours treatment at (B) 27°C, (C) 30°C and (D) 33°C

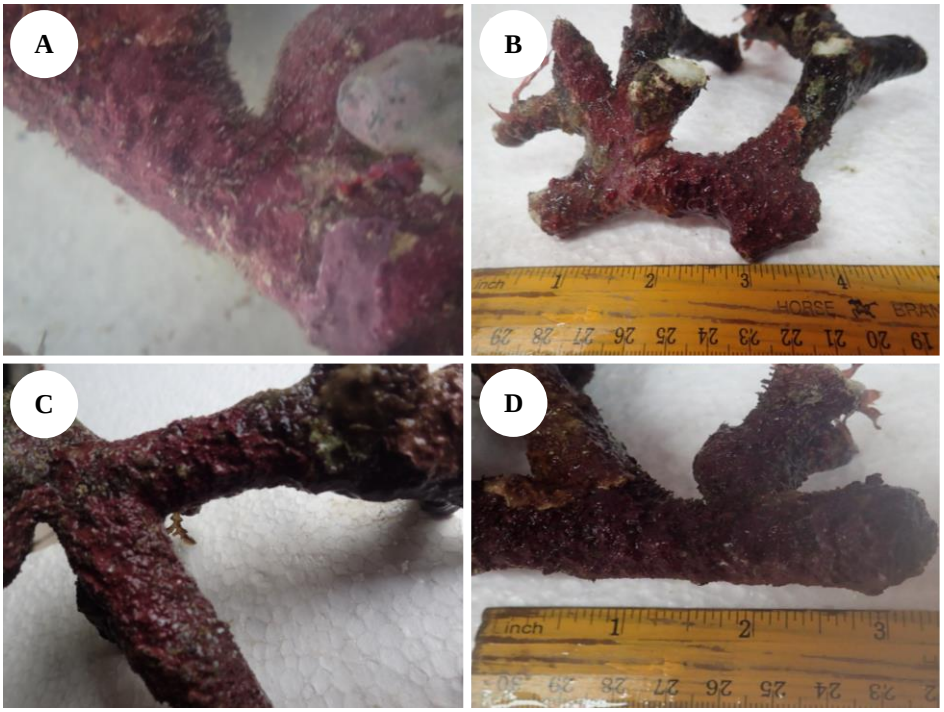

Figure 7. Visual observation of colors before and after thermal experimental trials on RCA *L. incrustans*. Color observed: (A) Healthy color before experiment, color following 19 hours treatment at (B) 27°C, (C) 30°C and (D) 33°C

Discussion

Distribution of Red Coralline Algae around Mauritius Island

Historically, research on RCAs in the Republic of Mauritius has been overlooked. Nevertheless, some work has been carried out on Mauritius's main island, for instance, by Ballesteros and Alfonso-Garrillo (1995). In the present study, non-geniculate RCAs that lack a non-calcified segment in the middle of the calcified segment were the most abundant species on hard substrate in all zones. Results indicated that areas covered by sand were most common in the near-coast zone of all studied sites and lagoon zones. Consequently, poor distribution of RCAs was observed in these zones, owing to loose substrate type. A similar observation was made by Lei et al. (2018), showing the influence of the sand substrate on the distribution of RCAs. All the species identified in Mauritius occur either on dead coral (broken and dead stand) or as epizoic (on dead or living gastropods). *Hydrolithon* has been found as epiphytes on seagrass in the lagoon area at BM. This supports the finding of several authors (Bramwell and Woelkerling 1984; Payri et al. 2001; Woelkerling et al. 1993) who have reported species of *Hydrolithon* to be common epiphytes on seagrasses in various regions worldwide. Lagoon and reef zones of almost all studied sites were observed with good live coral cover and coral rubble, thus, the distribution of RCAs was higher due to substrate type. Consequently, RCAs were widely distributed at the lagoon and reef zones at all studied sites with abundant hard substrates. Kroeker et al. (2013) reported a similar observation, demonstrating the importance of hard substrate availability for growth and distribution.

Non-geniculate RCAs dominated all studied sites in sheltered areas and exposed reef flats except at FEF. Geniculate RCAs were higher in all near-coast zones (5.85±41.00%), lagoon (2.66±59.10%) and reef (2.25±28.10%). Similarly, Ballesteros and Alfonso-Carillo (1995) reported that non-geniculate RCAs were dominant at Trou d'Eau Douce, Mauritius, overgrowing different hard substrate types. The highest distribution of RCAs was observed on hard substrate on *Acropora*, with genera *Hydrolithon* and *Lithophyllum* being the most dominant species. The *H. onkodes* was found to be abundant on dead over-turned tabular *Acropora*, and many coral recruits were observed. This supports the finding of several authors (Adey et al. 1982) who have reported *Hydrolithon* as the main reef-building RCA.

Sessile organisms' recruitment is a vital ecological process that impacts the organization and conservation of ocean communities by effectively spreading and settling movable gametes. Therefore, larval settlement is a crucial stage in the life cycle of various invertebrates, for instance, reef-building coral. Specific compounds induce coral larval settlement, for example morphogens (Gomez-Lemos et al. 2018). Documented three decades ago, it was revealed that RCA was a crucial group of benthic communities whose chemical compounds play an imperative role as inducers for marine invertebrates larvae, including corals. Therefore, the high abundance of non-geniculate RCAs at the different studied sites clearly indicates that natural regeneration will occur. However, any disruption to interaction is of particular concern to the recruitment success of coral. Coral recruits *Hydrolithon* spp. have well been observed at many studied sites, mainly BM in Mauritius. Similar findings were reported by authors (Birrell et al. 2008; Elmer et al.

2018), who have documented coral larval settlement and recorded to enhancement rate of settlement of larvae of coral on *Hydrolithon* spp. This research provides a geographically substantial assessment of the community structure of RCA in the east, west and north of Mauritius.

Knowledge of the different non-geniculate in Mauritius sustaining the mechanisms driving coral larvae-microbe-plant interaction and the influence of RCA on initial life history processes of coral larvae will enable a sound understanding of drivers of reef retrieval succeeding disruption.

Thermal tolerance experiment coralline red algae

The RCAs respond differently to PAM fluorometry techniques compared to other algae owing to the presence of phycobilisome in their photosynthetic apparatus (Burdett et al. 2012). The *L. incrustans* and *H. onkodes* are non-geniculate RCAs essential to the coastal ecosystem and functions. The laboratory thermal study results demonstrated the adverse effect of elevated temperature on RCA photo-physiology *L. incrustans* and *H. onkodes*; the latter was more affected among the two species studied. It was observed that raised temperature gave rise to continuous photodamage build-up, evidenced by a significant progressive decrease in effective quantum yield throughout the study in the two species. No build-up of photodamage was detected in the control treatment 27°C, suggesting that the experimental seawater situations were ideal, and the two RCAs were well photo acclimated to their corresponding temperature environments.

At temperature treatment 30°C PAM measurement demonstrated a continuous increase in photo-physiology for *L. incrustans*. This can be explained as a temperature-enhanced metabolic rate through increased enzyme activity. After exposure to temperature stress 33°C for 3 hours, PAM records demonstrated significant losses in effective quantum yield associated with photodamage enhancement, which was principally noticeable for the *H. onkodes*. Thus, *L. incrustans* has more thermal adaptation than *H. onkodes*. These findings indicated that heat-induced photodamage was significantly worsened under raised heat in *H. onkodes*. This result highlights the major role of heat stress in the gravity of the effect of global warming resulting in rising seawater temperature on RCA performance, consistent with former results documented for some RCAs. However, it is not necessarily signifying that ocean warming will have an unlimited impact on all RCA species, initially, certain species are eventually more susceptible than others. At 19 hours, the *H. onkodes* was bleached to pale purple, followed by necrosis and eventually death. This finding is in line with other authors reported that bleaching causes loss of photosynthetic pigment and, in several cases, may lead to necrosis (Martone et al. 2010) and an increase of temperature 2-4°C above the seasonal maxima causes approximately 90% bleaching. However, the outcome of upcoming climate change will depend on the ability of a species to adapt or acclimatize over various generations rather than short-term responses measured in nearly all laboratory research. The outcomes of this study not only highlighted the impact of rising seawater temperature due to global warming but reinforced the importance of non-geniculate communities in contributing to coral reef ecosystem maintenance by assisting in the regeneration of disrupted areas through enhancing coral larval settlement, particularly reef-building coral.

This investigation showed that some differences exist between species living under similar depth conditions and consequences of changes in temperature of the marine environment on *H. onkodes*. These findings have implications for coral recruitment on degraded reefs and need more attention to better manage the resilience and recovery of coral reefs.

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Photo-physiological responses and thermal tolerance of regionally endemic/rare and morphologically different corals of the Western Indian Ocean

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[1]Department of Biosciences and Ocean Studies, Faculty of Science and Pole of Research Excellence in Sustainable Marine Biodiversity, University of Mauritius, Réduit 80837, Republic of Mauritius. Tel./Fax.: +230-4037916, email: ♥vmunbodhe@gmail.com, ♥♥r.bhagooli@uom.ac.mu
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Abstract. *Munbodhe V, Jeetun S, Ricot M, Jogee S, Kaullysing D, Bhagooli R. 2023. Photo-physiological responses and thermal tolerance of regionally endemic/rare and morphologically different corals of the Western Indian Ocean. Indo Pac J Ocean Life 7: 100-107.* Intensification in climatic variations is causing major alteration in ecosystem functionalities and an overall decline in reef biodiversity. Underlying the ongoing cumulative threats and the vulnerability to biodiversity loss in the reefs, this study aims to determine the photo-physiological response and the thermal tolerances of the morphologically different coral species namely, *Porites lutea, Porites cylindrica, Acropora hyacinthus, Galaxea fascicularis, Seriatopora hystrix* including the two regionally endemics of the Western Indian Ocean, *Acropora branchi* and *Pocillopora indiania*. Coral fragments from three colonies per species were collected from the south and southeast of Mauritius Island and treated at 27°C, 30°C and 32°C for 19 hours. Using a diving Pulse-Amplitude-Modulated (D-PAM) fluorometer, the effective quantum yield at photosystem II (ΦPSII) was recorded from the coral fragments initially and following 3, 6 and 19 hours of treatment. This experiment determined the thermal threshold of the understudied *A. branchi* and *P. indiania*, and detected the unexpectedly enhanced thermal tolerance of *S. hystrix* and *G. fascicularis*. Overall, it provides a preliminary insight into potential thermal stress tolerance in some Mauritius corals and has shown that these corals might have strategized to enhance their thermo-resilience while others are still struggling to withstand such stresses. These findings on the thermal resilience of regionally endemic/rare and morphologically different coral species are essential for further reef conservation efforts and the selection of coral species for reef restoration.

Keywords: Coral morphology, photo-physiological response, regionally endemic corals, thermal stress, thermal tolerance

INTRODUCTION

Compounding effects from both climatic variabilities and massive disturbances arising from the ever-increasing anthropogenic activities have resulted in immense loss of the 3-dimensional structure of the reefs (Elliott et al. 2018; Richardson et al. 2018). Warm sea surface temperatures have seriously damaged and killed up to 16% of the world's coral reefs (Steffen et al. 2009) and under the current warming scenario (Klepac et al. 2022), coral reefs are expected to undergo further major benthic transformation (van Hooidonk et al. 2015). Australia's Great Barrier Reef has already experienced this phenomenon eight times, causing mass bleaching since 1979 with the most widespread events occurring in 1998 and 2002 whereby more than 50% of reefs bleached (Steffen 2009). Likewise in the Caribbean, about 80% of the reefs was affected in 2005, while since 2014, three major bleaching events were recorded, with 2014 in the Northern tropical Pacific, 2015/16 over the tropical oceans (Lough et al. 2018) and 2017/18 in the Western Indian Ocean (WIO) (Obura et al. 2019).

To understand the mechanism of coral bleaching, Pulse-Amplitude-Modulated (PAM) fluorometry has been used to measure changes in photosynthetic activities and the response of corals to increasing sea surface temperatures as well as to changes in light intensity with increasing depth (Bhagooli and Hidaka 2003; Hoogenboom et al. 2012; Louis et al. 2016; Bhagooli et al. 2021a). The rate of bleaching varied significantly in different coral morphs with increasing degrees of temperature, for example, in *Acropora selago*, the expulsion mean number of degraded cells from corals increased from 36 degraded cells at 27°C to 60 degraded cells at 30°C and 54 degraded cells at 27°C to 75 degraded cells at 30°C in *Acropora muricata* (Fujise et al. 2014). Furthermore, photo-physiologically, not all corals react in the same manner (Bhagooli and Hidaka 2003; Fitt et al. 2009; Tilstra et al. 2017; McWilliam et al. 2018; Serrano et al. 2018; Mattan-Moorgawa et al. 2020) to severe and frequent prolonged marine heating (Hobday et al. 2016; Stillman 2019). Some suggest that corals from highly diverse coral reefs are more thermotolerant with relatively high productivity (McWilliam et al. 2018). Others infer that varying size and shape of the corals may respond differently (van Woesik et al. 2011) whereas corals

exposed to vast thermal variabilities tend to show more thermal resistance than those from thermally stable environment (Louis et al. 2016, 2020; Safaie et al. 2018; McClanahan and Muthiga 2020; Voolstra et al. 2020).

Furthermore, it has been reported that local factors such as over-fishing, nutrient enrichments (Serrano et al. 2018), and sedimentation (Shantz and Burkepile 2014) contributes significantly to algal over-growth, thereby compromising the resilience of corals by making them more vulnerable to thermal bleaching (Holbrook et al. 2018). Under certain conditions, Baird and Marshall (2002) successfully provided evidence that the corals of the same genera exhibited different reaction to the thermal stress whereby, *Acropora millepora* was not only more resistant than *A. hyacinthus* but also remarked that certain colonies of the same *Acropora* species survived the entire bleaching event while others died. Moreover, despite elevated temperature, *Stylophora pistillata* and *Galaxea fascicularis* could tolerate thermal stress under favorable food conditions and thus sustain symbiotic photosynthetic activities implying thermal adaptability (Borell and Bischof 2008).

Yet, lethal coral bleaching has been increasing in frequency, severity, and geographic scope over the past decades, and thus it is anticipated that this trend will continue (Grottoli et al. 2014; Hughes et al. 2017) which therefore increases the fragility of reefs (Goldberg and Wilkinson 2004) leading to biodiversity loss (McClanahan et al. 2021) and rapid community structural changes (Tebbett et al. 2023). To date studies on corals of Mauritius have looked at bleaching and mortality of corals under thermal stress (Bhagooli and Taleb-Hossenkhan 2012; Bhagooli and Sheppard 2012; Bhagooli and Kaullysing 2019; Bhagooli et al. 2021b,c; Fai et al. 2023; Jeetun et al. 2023; Ricot et al. 2023). However, rare and endemic corals' responses to thermal stress in the Mauritian waters is almost uncharted. Underlying the ongoing cumulative threats and the vulnerability to biodiversity loss, this study examines whether the degree of thermal stresses vary with respect to coral growth-forms, branching, tabular, massive and sub-massive corals and assess the thermal threshold of the under-studied regionally endemic coral species. Overall, it aims to elucidate impact of the increasing severity of thermal stresses and determine the photo-physiological responses and the thermal tolerances of the morphologically different coral species including the two regionally endemics of the WIO.

MATERIALS AND METHODS

Study area

The Republic of Mauritius is an oceanic state in the Western Indian Ocean which has several outer islands harbouring highly diverse corals and reef fishes (Obura 2012). Mauritius, the main island of the Republic of Mauritius, has an overall lagoon area (shallow waters) of 243km^2 enclosed by about 150 km of fringing reefs. The reefs are distinctly broken by natural breaks giving rise to a series of lagoons that defines the geomorphological characteristics such as depths, current pattern and stretches from the shore (Daby 2006). The depth of the lagoons varies between <1 to 3m from near shore extending a few kilometres to the reef with some exceptions in the southeast region where channels of < 30m deep mainly associated with rivers or underground springs separate the reef from the shore (Elliott et al. 2018). Serious concerns on the decadal increase in atmospheric temperature at the rate of 0.5°C per decade were raised following the metadata analysis which had shown that weather condition in Mauritius is experiencing a mean highest temperature of 31°C in summer and mean lowest temperature of 14°C in winter (Boojhawon et al. 2010). In addition, the reefs of Mauritius are also exposed to ongoing coastal clearing and intense near-coast and inland urbanization (Elliott et al. 2018), making them more vulnerable to unprecedented risks of local extinctions (Obura et al. 2017; McClanahan and Muthiga 2020).

Subsequently, this study was designed to assess the thermal tolerance of *Acropora branchi* and *Pocillopora indiania*, the regionally endemic corals along with *Acropora hyacinthus*, *Porites lutea* and *Porites cylindrica* and *Seriatopora hystrix*. These coral specimens were collected in the form of 2-2.5cm^2 fragments from different coral colonies of the same species from the backreef and mid-lagoon of Riviere des Galets from the South of the Island, whereas *S. hystrix* fragments which are among the rare coral species were collected from Bambous Virieux, mid-lagoon which is situated in the eastern part of Mauritius (Figure 1). Both lagoons are continuously exposed to the South East Trade winds with a water depth varying from 0.75m to 1.5m at the high tide. The *A. branchi, A. hyacinthus, P. indiania, P. lutea* and *P. cylindrica* were relatively abundant at Riviere des Galets, 20° 30.706'S; 57° 27.903'E while *S. hystrix* was found in the eastern part of the island at Bambous Virieux, 20° 20.925'S; 57° 46.057'E.

Thermal stress experiment

Based on the occurrences, in February 2022 coral fragments were collected from the two selected sites and were brought in buckets under dim light to Environmental Sciences Wet Laboratory at the University of Mauritius. The fragmented samples were transferred to 2000L tanks equipped with air pumps for at least two hours of settling before the thermal stress experiment. Nine coral fragments (n=9) were used during the experiment, whereby three coral fragments per coral colony of the same species were placed in three different aquaria of the same treatment (triplicates), whereby nine sets of photophysiological readings from each treatment were obtained. The nine aquaria (400-500L) were equipped with air pumps and thermostats to maintain the dissolved oxygen level to an optimal level and maintain the water temperature to the desired experimental level. The initial temperature in all 9 tanks was about 27°C (normal SST) and salinity at ~35 ppt. Three aquaria (3x Tank 1) were used as Control while the 6 others were used for bleaching with temperature gradually raised to 30°C (thermal threshold) in aquaria Experimental Tank 2 and 32°C (induced stress) in aquaria Experimental Tank 3 (Figure 2).

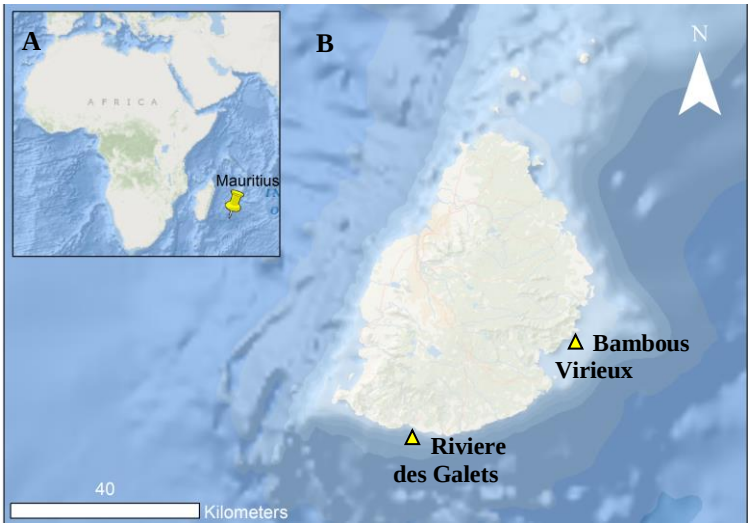

Figure 1. A. Location of Mauritius in WIO indicated by yellow pin, B. Sites where coral samples were collected; Riviere des Galets; bleaching surveys were carried out in January and April 2019 and coral fragments from six coral species were collected for thermal stress experiment while only fragments from one species from Bambous Virieux was collected i.e., *Seriatopora hystrix* which is relatively rare and occurrence is site specific

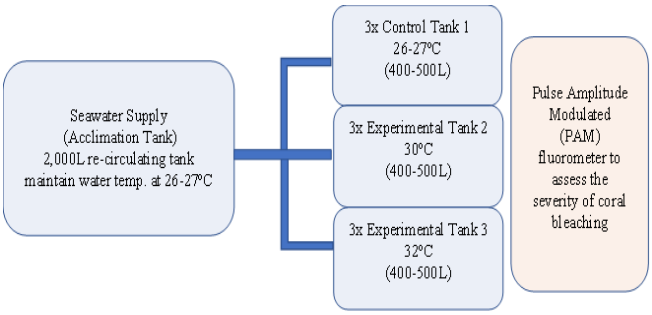

Figure 2. Experimental design for thermal stress using three different treatment tanks at 27°C, 30°C and 32°C with each treatment having three experimental tanks (triplicate) and each experimental tank having three coral fragments of the same species (3 coral fragments x 3 experimental tanks per treatment, n=9 per coral species)

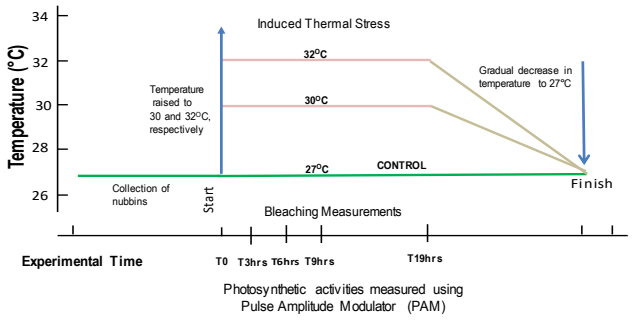

Figure 3. Conceptual diagram showing the different steps involved in the thermal stress experiment from the collection of coral fragments to exposure to different temperatures and the timely measurement of photosynthetic activities

Prior to placing the coral fragments in their respective treatment tank, photo-physiological data was recorded at the beginning of the experiment, i.e., $Time_{initial}$ or $Time_{zero}$ was collected prior to placement of the coral fragments in each water tank using the Pulse-Amplitude-Modulated (PAM) Fluorometry (Bhagooli and Hidaka 2003; Bhagooli et al. 2021a). Same exercise was repeated at three hours, six hours and 19 hours as demonstrated by the conceptual diagram (Figure 3).

Data analysis

MS Excel and R-Statistical software, R-4.2.2 were used to compile and analyse the effective quantum yield, Φ_{PSII}. The bar-plots were used to display thermal stresses, Φ_{PSII} of each coral species following persistent exposure to varying temperatures under experimental conditions. The percentage reduction in the photosynthetic response (relative to initial) was derived from the mean effective quantum yield of each coral species to that of initial mean Φ_{PSII} for every treatment. As the data on the Φ_{PSII} was not normally distributed (Shapiro-Wilk Test for normality), non-parametric statistical analysis was performed to assess the impact of elevated temperature on the Φ_{PSII}. Kruskal-Wallis Test shed light on the variation in photosynthetic activities combined for all coral species with respect to mean effective quantum yield (Φ_{PSII}) at the different treatments over time, while the Dunn's (1964) Kruskal-Wallis Post-hoc test allowed multiple comparison of the mean effective quantum yield (Φ_{PSII}) at different treatments after 3 hours, 6 hours and 19 hours of thermal stress experiment.

RESULTS AND DISCUSSION

Thermal stress experiment

The thermal stress experiment showed that *A. branchi* (Φ_{PSII} = 0.007±0.001 at 30°C and ΦPSII = 0.006±0.001 at 32°C), *A. hyacinthus* (Φ_{PSII} = 0.11±0.04 at 30°C and Φ_{PSII} = 0.00 at 32°C) and *P. cylindrica* (Φ_{PSII} = 0.009±0.003 at 30°C and Φ_{PSII} = 0.02±0.03 at 32°C) were photo-physiologically inactive after 19hrs exposure to 30°C and

32°C, respectively. The coral fragments of *A. branchi*, *A. hyacinthus* and *P. cylindrica* were completely bleached while, after persistent exposure, *P. lutea* (Φ_{PSII} = 0.476±0.008 at 30°C and Φ_{PSII} = 0.357±0.007 at 32°C), *G. fascicularis* (Φ_{PSII} = 0.0.463±0.067 at 30°C and Φ_{PSII} = 0.164±0.007 at 32°C and *S. hystrix*, (Φ_{PSII} = 0.677±0.003 at 30°C and Φ_{PSII} = 0.185±0.081 at 32°C) exhibited relatively higher photosynthetic activities. Conversely, *P. indiania* was photo-physiologically active after 19hrs of persistent exposure to 30°C, (Φ_{PSII} = 0656±0.05 at 30°C) with tissue blushing and paling/partial bleaching which eventually could not resist higher thermal stress of 32°C at 19hrs (Φ_{PSII} = 0.002±0.002 at 32°C).

Overall, the photosynthetic activities remained at maximum within the first 3 hours of the experiment which then gradually varied significantly at 6 hours and at 19 hours ($p<0.001$, Table 1). However, at 6 hours, no differences in photo-physiological activities was observed in coral fragments exposed at temperatures 27°C and 30°C ($p=0.266$) as compared to 27°C and 32°C and 30°C and 32°C which differed significantly ($p<0.0001$, Table 2). Relatively only about 10% drop in the effective quantum yield in corals was recorded during the first 6-hrs at 30°C which intensified after persistent exposure (19 hours) whereby the photosynthetic activities of *A. branchi*, *A. hyacinthus*, and *P. cylindrica* were ceased completely while *P. indiania*, maintained 80% - 90% of its photo-physiological activities both at 30°C/19 hours and 32°C/6 hours whereas complete bleaching was observed at 32°C/19 hours as depicted in the bar-plots (Figure 4). Most of the corals exhibited 60% (relative to initial) of photo-physiological response to 32°C within the first 6 hours of the experiment. However, only *P. lutea, G. fascicularis* and *S. hystrix* were capable to maintain photo-physiological response to persistent higher temperature of 32°C for 19 hours at a rate of 47%, 22% and 23%, respectively (Figure 5), while the rest of the coral fragments were completely bleached and smothered.

Table 1. Kruskal-Wallis Test: Variation in photosynthetic activities combined for all coral species with respect to mean effective quantum yield (Φ_{PSII}) at the different treatments over time; $p<0.01$, sig. *, $p<0.001$, sig. **, $p<0.0001$, sig. ***

Experiment duration	Chi-squared	d.f.	p-value
3 hours exposure	7.249	2	0.026
6 hours exposure	30.282	2	<0.0001
19 hours exposure	36.696	2	<0.0001

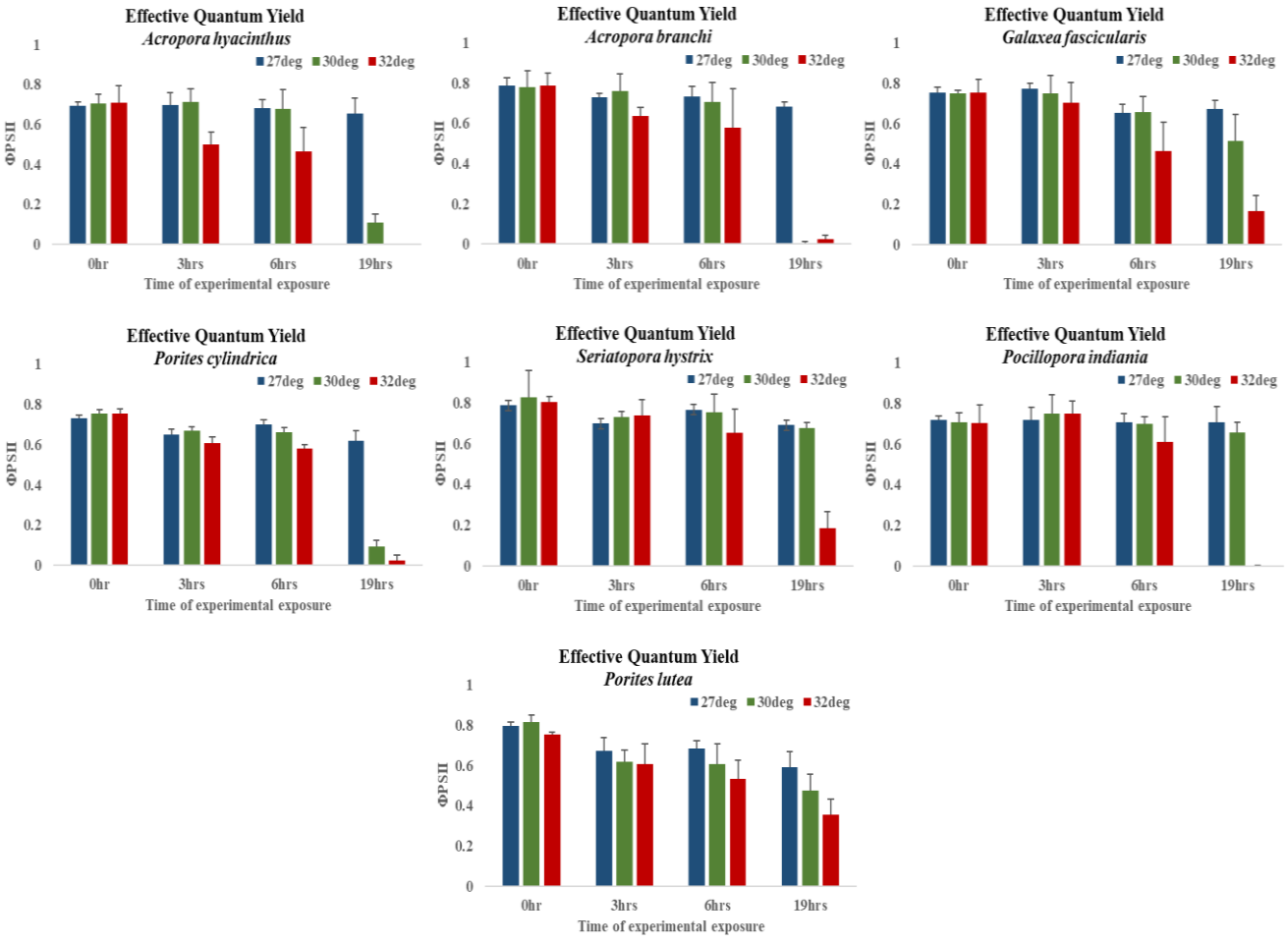

Figure 4. Effective quantum yield at PSII (ΦPSII) of seven coral species at temperatures 27°C (Control), 30°C and 32°C under experimental conditions. The photosynthetic activities were recorded at beginning ($T_{initial}$) of the experiment, after 3 hours of the experiment (T_{3hrs}), after 6 hours of the experiment (T_{6hrs}) and after 19 hours of exposure (T_{19hrs}). Data represents mean±SD (n=9)

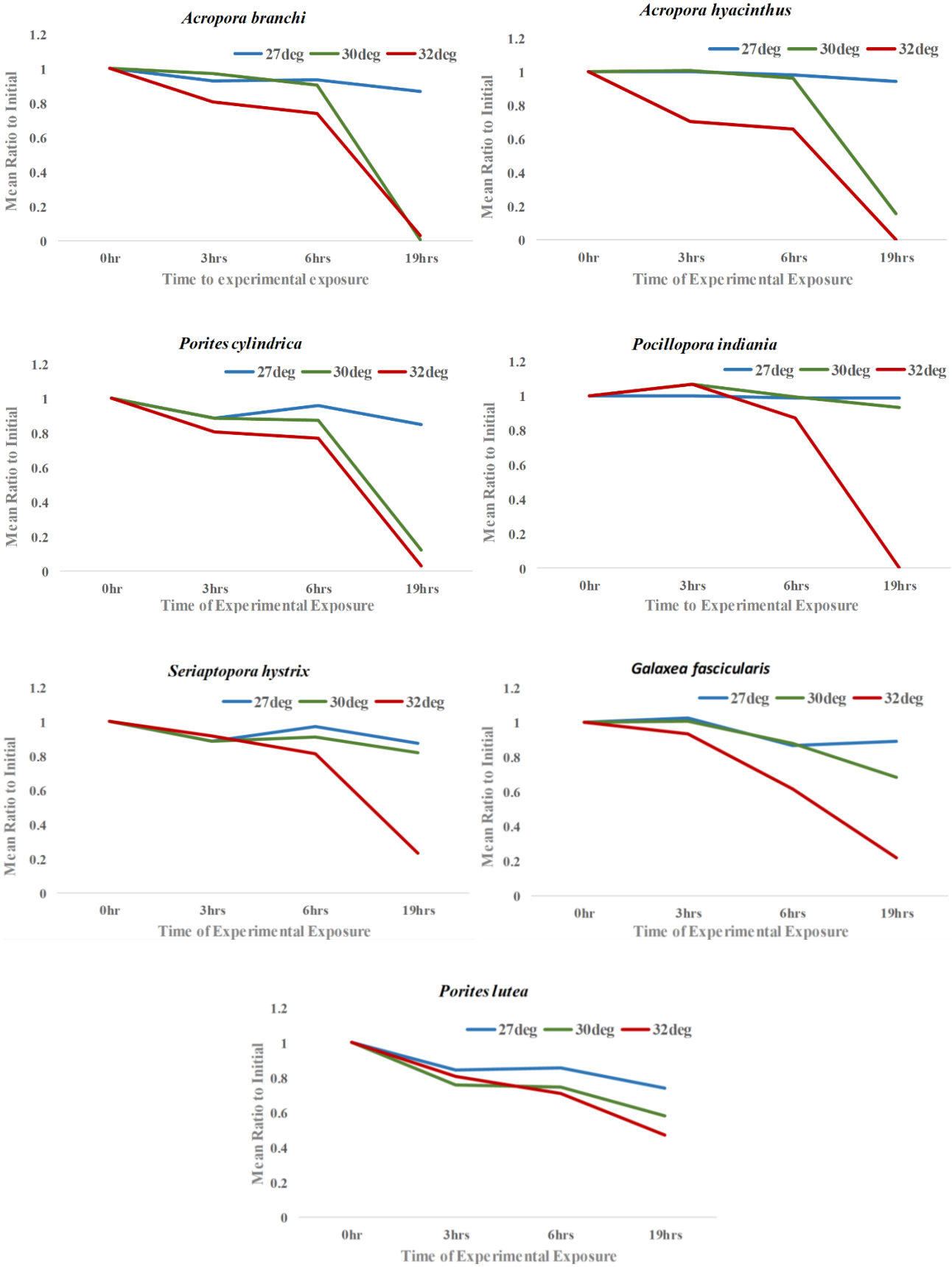

Figure 5. Relative (to T_{0hr}, i.e. initial) effective quantum yield of 7 tested corals exposed to 27°C, 30°C and 32°C over 19hrs

Table 2. Dunn's (1964) Kruskal-Wallis Post-hoc test: Multiple comparison of the mean effective quantum yield (Φ_{PSII}) at different treatments after 3 hours, 6 hours and 19 hours of thermal stress experiment; p<0.01, sig. *, p<0.001, sig. **, p<0.0001, sig. ***

Treatment	3 hours exposure			6 hours exposure			19 hours exposure		
comparison	Z	p-unadj.	p-adj.	Z	p-unadj.	p-adj.	Z	p-unadj.	p-adj.
27°C - 30°C	-0.408	0.683	0.683	1.116	0.265	0.266	3.284	0.001	0.001
27°C - 32°C	2.101	0.036	0.071	5.224	<0.0001	<0.0001	6.051	<0.0001	<0.0001
30°C - 32°C	2.509	0.012	0.036	4.109	<0.0001	<0.0001	2.766	<0.0001	<0.0001

Discussion

This laboratory-based study established the thermal threshold of two understudied regionally endemic corals, *A. branchi* and *P. indiania*, along with five other morphologically different corals. Overall, the corals displayed a varying degree of thermal tolerances concerning persistent exposures (19 hours) to thermal stresses at 30 and 32°C. Furthermore, it was found that tabular *A. branchi*, *A. hyacinthus* and branching *P. cylindrica* were the most susceptible to bleach and die after 6 hours of exposure to a persistent warmer environment. On the other hand, *P. lutea*, *G. fascicularis*, *S. hystrix* and *P. indiania* exhibited relatively higher thermal tolerance and these observations concurred with the findings made by van Woesik et al. (2011), Mattan-Moorgawa et al. (2012), Swain et al. (2016) and Cheal et al. (2017). Also, previous studies have demonstrated that tabular Acropora displayed signs of highest level of thermal stresses (Eriksson et al. 2012; Muko et al. 2013) while branching *Acropora* spp. and *Stylophora* spp. (Fitt et al. 2009) were considered more susceptible to bleaching and were the first to bleach and die compared to massive and encrusting corals such as *Porites* spp., *Leptastrea* spp., and *Goniastrea* spp. (Loya et al. 2001; Starko et al. 2023).

Only after three hours of exposure to 32°C, signs of thermal stresses were observed in *A. branchi, A. hyacinthus* and *P. cylindrica* fragments which were expressed by partial bleaching or paling of the coral fragments. Within six hours, the paling further intensified, increasing turbidity due to excessive mucus secretion compared to the 27°C treatments. All the treatments at 32°C had mucus but which varied considerably depending on the species of the coral fragments. Even the treatments containing the fragments of *P. lutea, G. fascicularis, S. hystrix* and *P. indiania* had accumulated mucus however, it was visibly lower in concentration. In this line, mucus usually acts as a primary defensive mechanism against microbial invasion (Ritchie 2006; Walters et al. 2020) as well as control their microbiome (Walters et al. 2020). Nonetheless, it has also been suggested that during thermal stress, nearly 45% of the total energy budget is concentrated in mucus production, which might be physiologically advantageous for thermally resistant corals as compared to the vulnerable ones as a consequence of further disadvantageously compromising the coral health (Fitt et al. 2009; Zaneveld et al. 2016). Studies have also demonstrated that thermal stresses may cause a reduction in the coral defense mechanism (Vidal-Dupiol et al. 2014; Beatty et al. 2019), while in-depth study on Acroporids revealed that coral immunity is also dependent on the reef health status, whereby the dominant algal reef has a higher risk of coral diseases due to pathogenic bacterial accumulation (Sandin et al. 2008; Louis et al. 2020; Beatty et al. 2022). As such, experimentally, it may be determined that one of the reasons for low thermal resilience in *A. branchi*, *A. hyacinthus* and *P. cylindrica* might have been excessive mucus secretion.

As expected, P. lutea exhibited higher thermal resistance than *G. fascicularis* and *S. hystrix* by actively demonstrating photo-physiological processes to persistent exposure to 32°C. The slow-growing, massive *P. lutea* is among the most studied massive coral and is well known for its thermotolerance (Hoogenboom et al. 2017; Hughes et al. 2018) compared to *G. fascularis* and *S. hystrix*. Despite being previously listed as thermally vulnerable (Loya et al. 2001), under the current laboratory conditions, *G. fascularis* and *S. hystrix* have shown certain level of thermal resistance most potentially owing to enhanced bleaching resilience strategies over repeated heating events (Hoey et al. 2016; Epstein et al. 2022). Given the fact that this study focused mainly on photo-physiological observations, the prediction of precise conditions favoring such thermotolerance was limited, nevertheless, such thermal adaptation may be associated primarily to the high diversity and reef complexity with low water residence time at Riviere des Galets (pers. observation), the sampling site for *G. fascicularis*. For example, certain corals are now capable of withstanding elevated temperatures (>2°C) provided that they have enhanced resilience through dynamic host-symbiont communities (Ziegler et al. 2018) by reshuffling of Symbiodiniaceae (Bhagooli and Hidaka 2003; Fitt et al. 2009; Guest et al. 2012) heterotrophy (Grotolli et al. 2006), shade from algal assemblages giving rise to photo acclimatization most potentially in the case of *S. hystrix* (Lewis et al. 2022) which are essential adaptive criteria for local thermal resistance and acclimatization.

So far, despite being limited to the assessment to only photosynthetic activities of morphologically different corals, this study has been able to assess the thermal threshold of the understudied *A. branch*i and *P. indiania*, the regionally endemic corals of the Western Indian Ocean region. Additionally, the degree of thermal vulnerability of *A. hyacinthus* and the thermal resistance of *P. lutea* was confirmed whereas the unexpectedly enhanced thermal tolerance of *S. hystrix* and *G. fascicularis* was detected. This experiment provided a preliminary insight on the potential thermal tolerance in some corals of Mauritius and

has shown that these corals might have strategized to enhance their thermo-resilience (Mattan-Moorgawa et al. 2020) while others in the like of tabular and branching ones are still struggling to withstand thermal stresses (Hoey et al. 2016). Also, such findings on thermal resilience of regionally endemic and morphologically different coral species are essential for onwards reef conservation and also for the selection of robust coral species for reef restoration. Nevertheless, in-depth evaluation through additional experimental parameters on these corals, such as the maximum Electron Transport Rate (ETRmax), Non-Photochemical Quenching (NPQ), study on heterotrophic feeding, microbiomes and Symbiodiniaceae community structure, is critical to providing science-driven information on photoacclimation and photoprotection on the reefs of the Western Indian Ocean region to better strategize on innovative conservation measures.

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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.

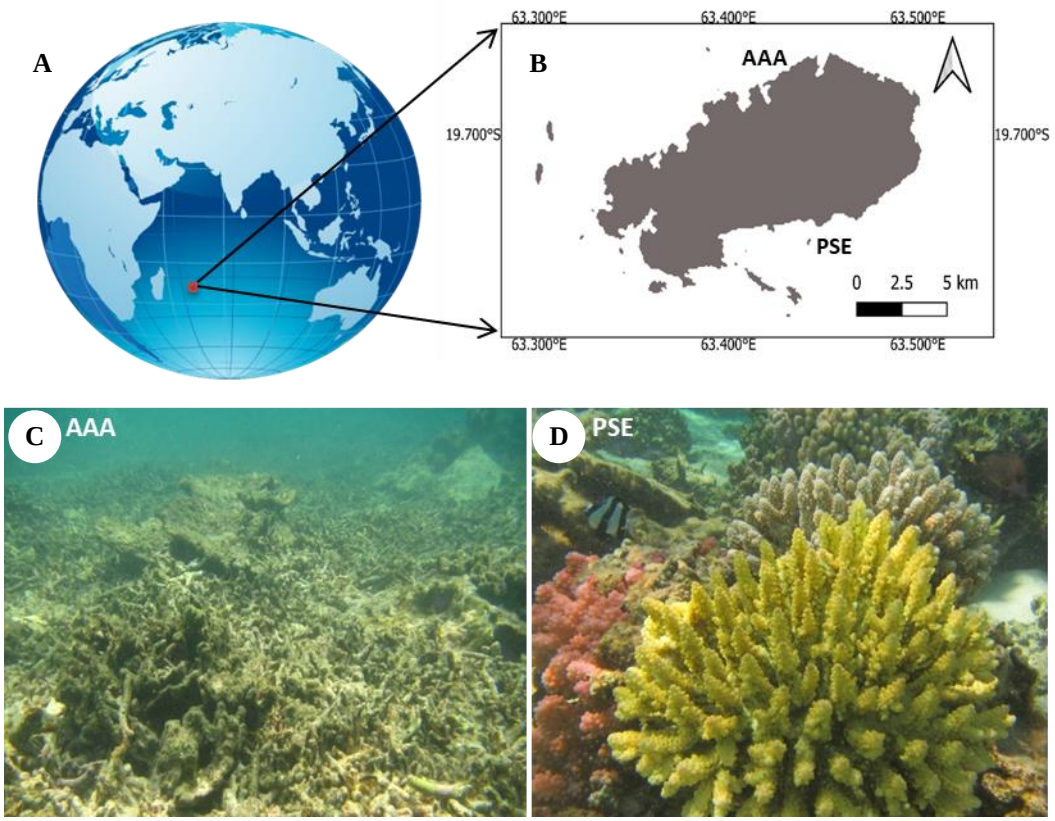

Figure 1. A. The World's globe with the red dot representing the study site in the Southern Indian Ocean, B. is the map of Rodrigues Island, Republic of Mauritius with the studied stations' AAA (Anse Aux Anglais) -Degraded reef and PSE (Port Sud-Est) - Non-degraded reef, C. Degraded reef at AAA and D.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

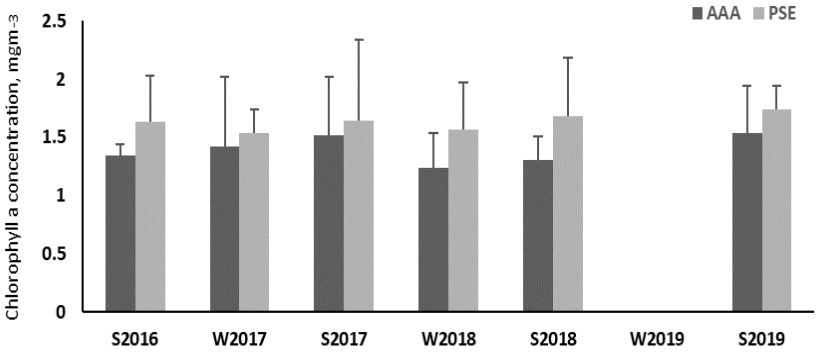

Figure 2. In situ chlorophyll-*a* concentration at Anse Aux Anglais (AAA) and Port Sud-Est (PSE) from summer 2016 (S2016) up to summer 2019 (S2019) in Summer (S) and winter (W) samplings

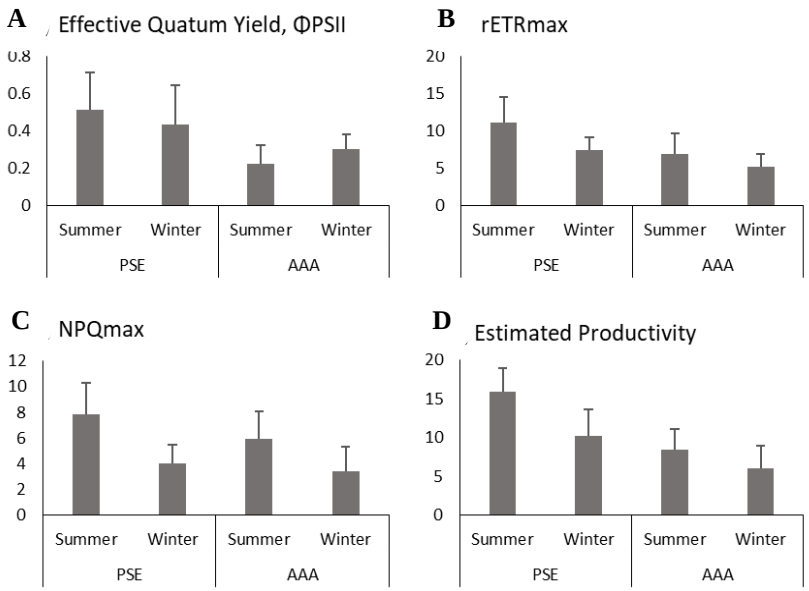

Figure 3. Photo-physiological parameters (y-axis): (A) Effective quantum yield (ΦPSII), (B) rETRmax, (C) npqmax and (D) Estimated Productivity, where the x-axis represent the sites, PSE and AAA, during summer and winter 2018

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).

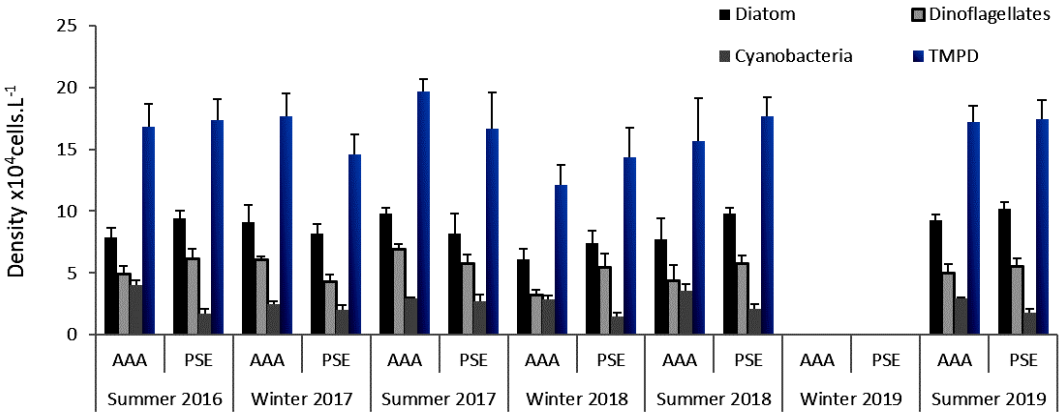

Figure 4. The densities of the TMPD (Total Micro-Phytoplankton Density), diatom, dinoflagellates, and cyanobacteria at the sites AAA (Anse Aux Anglais) and PSE (Port Sud-Est) from summer 2016 up to summer 2019 in the absence of the winter 2019 sampling

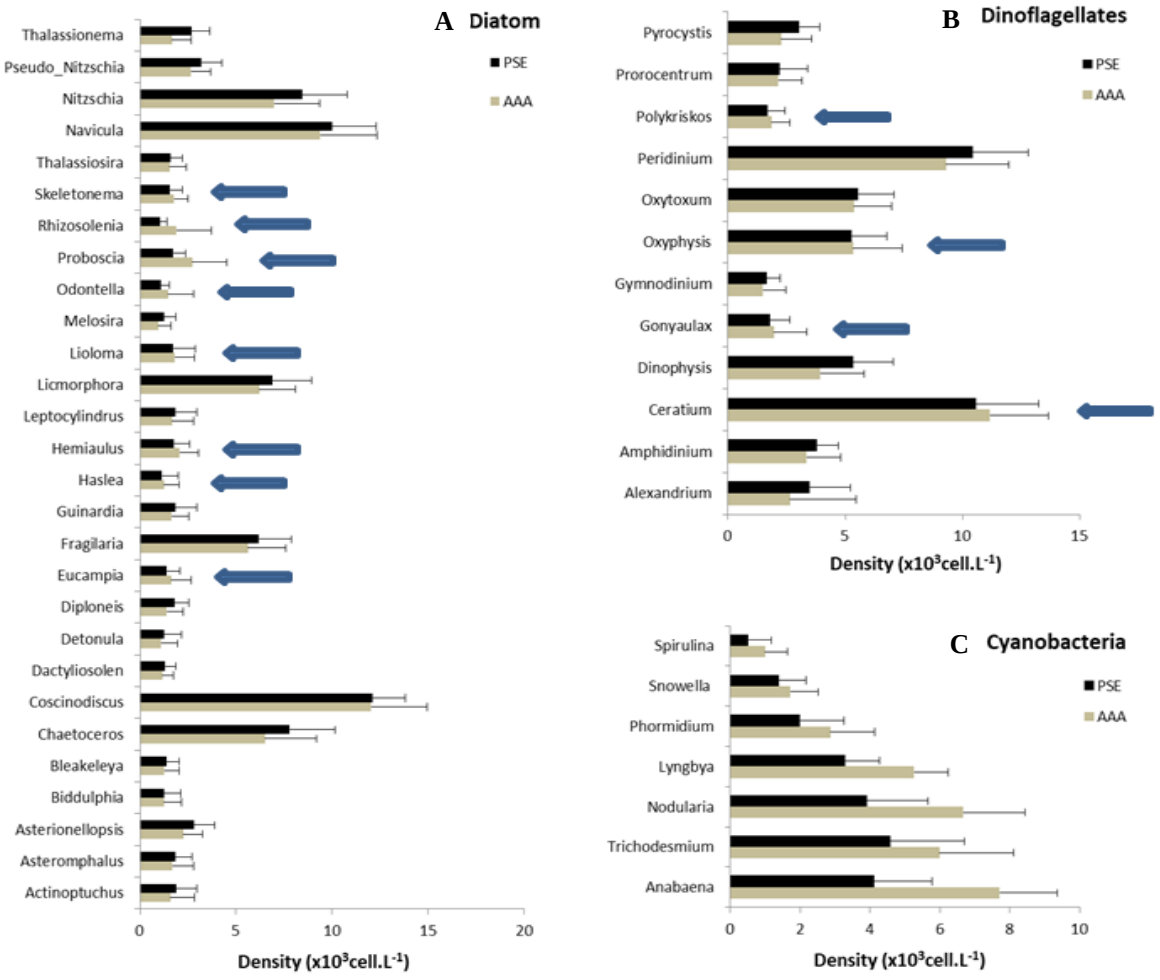

Figure 5. Densities of the 47 genera of micro-phytoplankton at PSE and AAA: (A) 28 genera of diatoms, (B) 12 genera of dinoflagellates, and (C) 7 genera of cyanobacteria. The blue arrows indicate the genera where the densities were higher at AAA compared to PSE

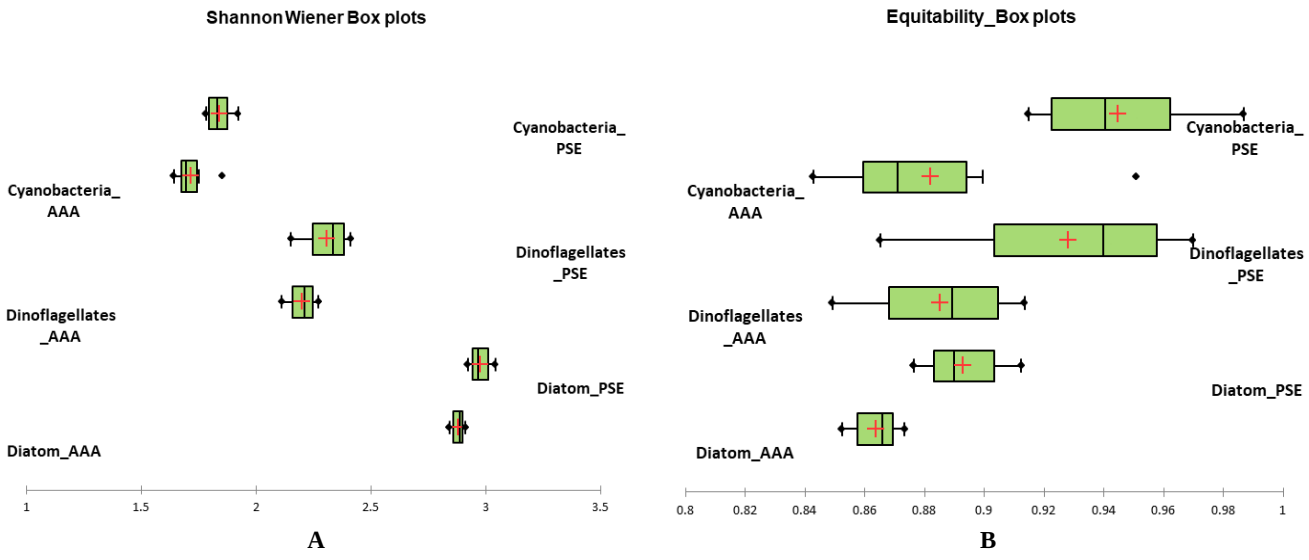

Figure 6. Representation of box plots for the diversity indices of the biological parameters diatom, dinoflagellates, and cyanobacteria at Anse Aux Anglais (AAA) and Port Sud-Est (PSE) where (A) is the Shannon Wiener (H') and (B) Equitability (EH)

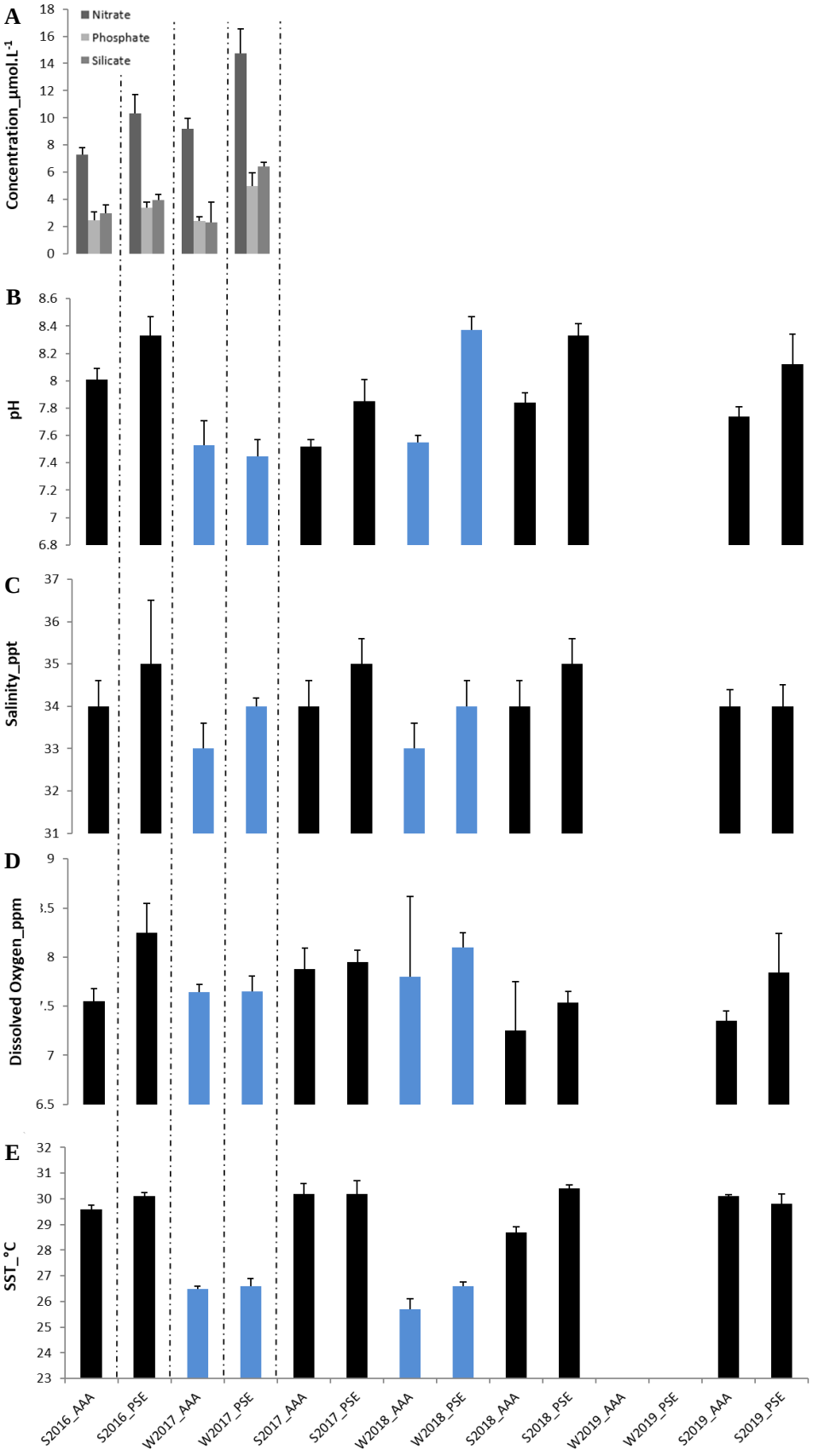

Figure 7. (A) Nutrient and (B-E) physico-chemical parameters variation during the alternate summer and winter season from 2016 up to 2019 at Anse Aux Anglais (AAA) and Port Sud-Est (PSE). The blue bars represent the winter and the black bars, summer for (B) ph, (C) Salinity, (D) Dissolved Oxygen, and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

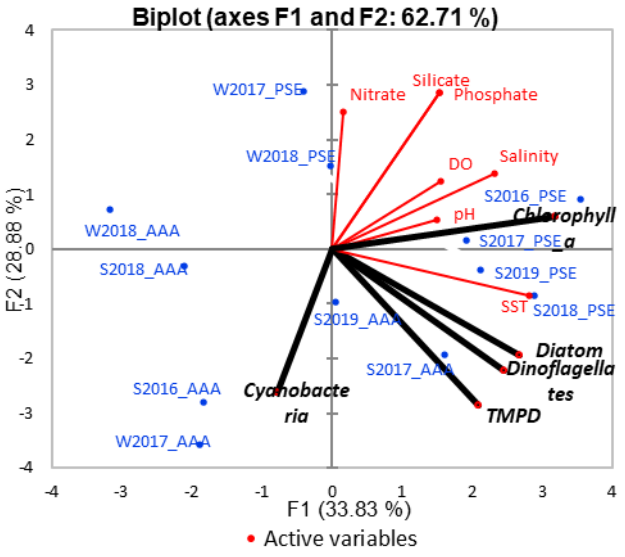

Figure 8. Principal Component Analysis (PCA) biplot analysis of the different physico-chemical parameters (nitrate, phosphate, silicate, chlorophyll-*a*, and SST-Sea Surface Temperature) and the biological parameters TMPD, diatom, dinoflagellates, cyanobacteria, and chlorophyll-*a* during the study period 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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