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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).

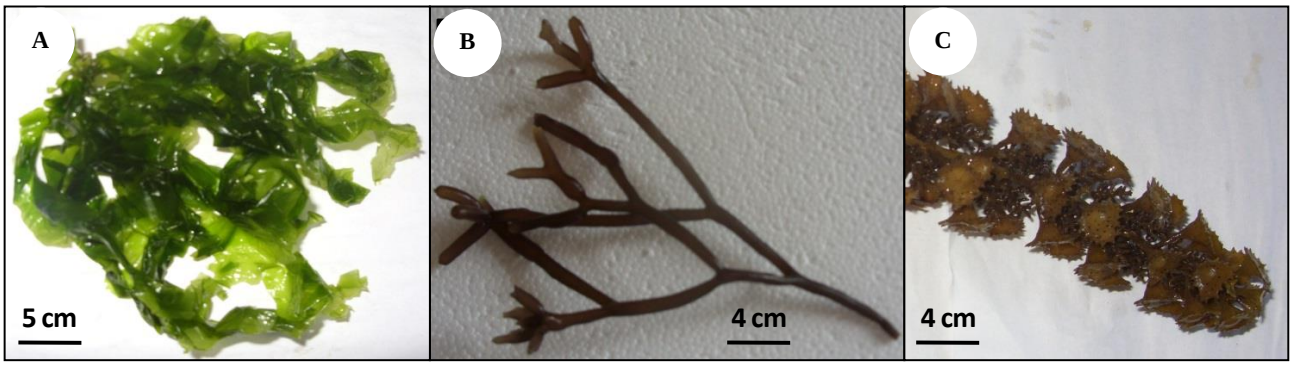

Figure 1. The three marine macroalgal test species A. *Ulva lactuca*, B. *Gracilaria salicornia*, C. *Turbinaria ornata*

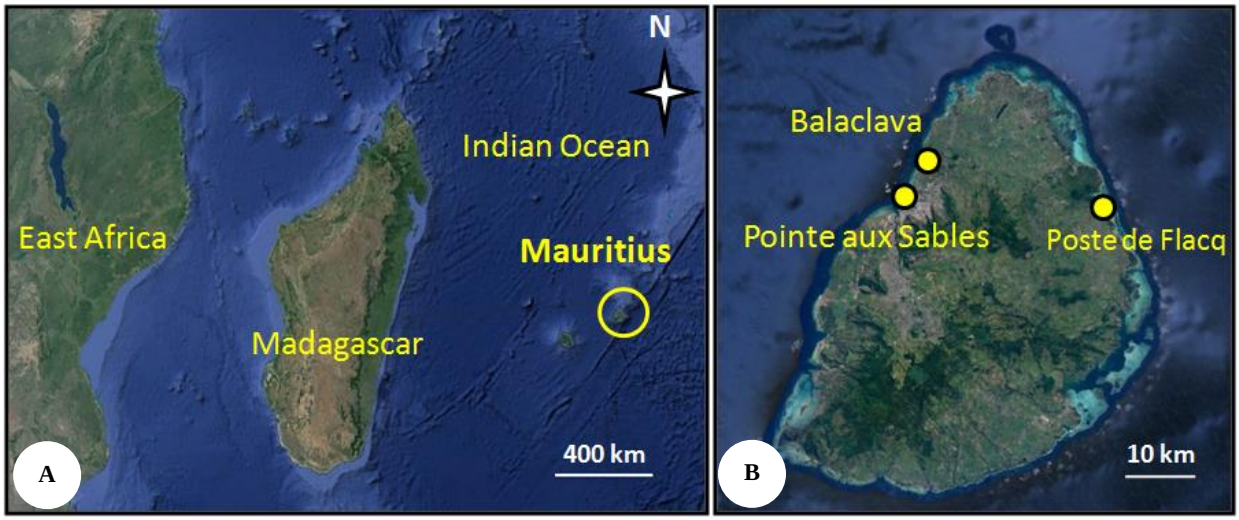

Figure 2. Satellite images of sampling sites. A. Mauritius Island is located in the South West Indian Ocean and B. The three macroalgal species, *U. lactuca*, *G. salicornia* and *T. ornata* were collected from different geographic regions of the Mauritius (Pointe aux Sables S20º10'13.2"E057º26'51.6", 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

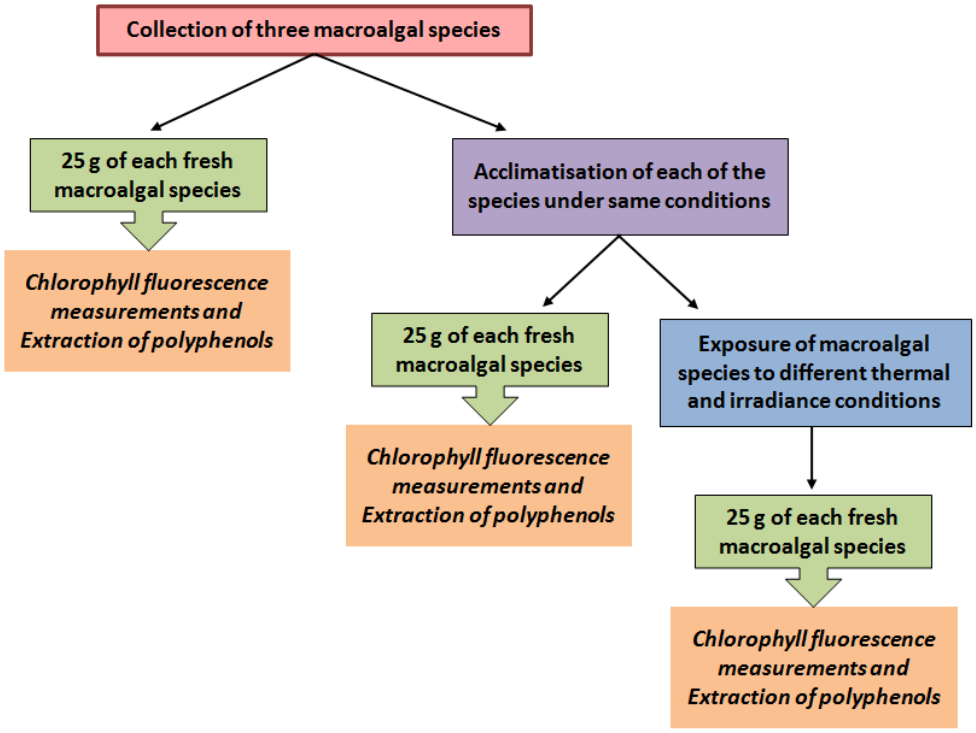

Figure 3. Flowchart representing the post-sampling treatments of macroalgal species and the intervals at which chlorophyll fluorescence measurements were taken and extraction of polyphenols was performed

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.

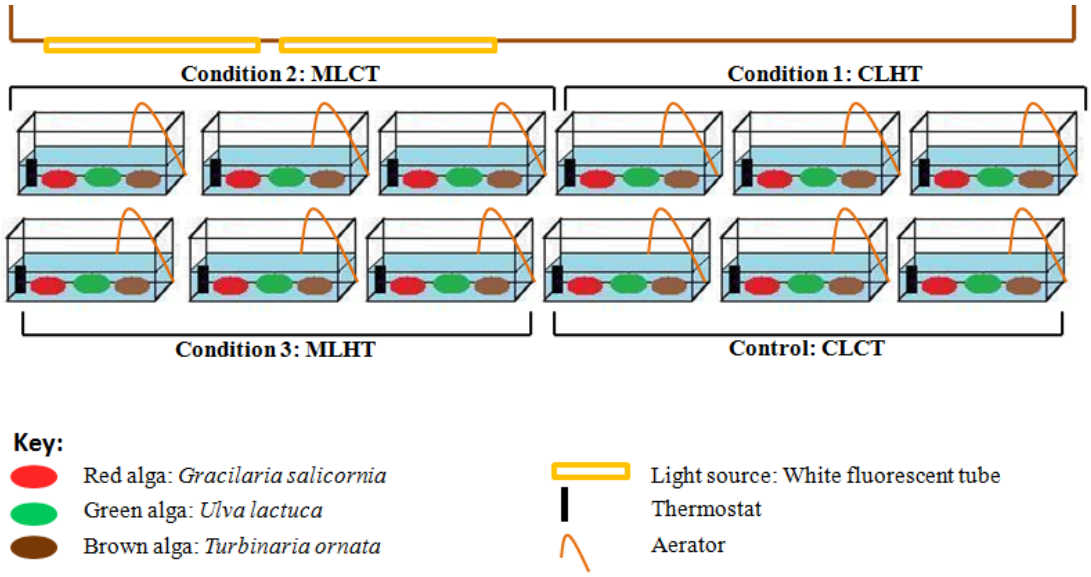

Figure 4. Diagrammatic representation of the experimental set-up used for application of temperature and light treatments on the three macroalgal species

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.

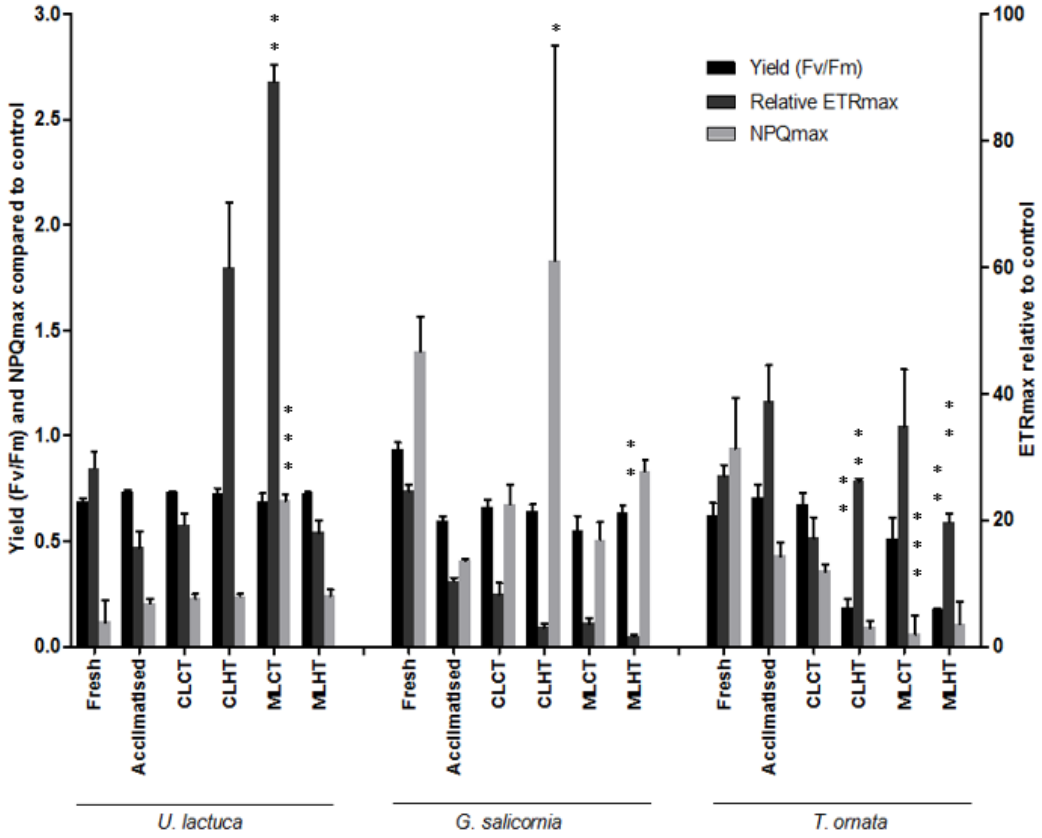

Figure 5. Photosynthetic performance in terms of chlorophyll fluorescence measurements: Fv/Fm(yield), rETRmax and NPQmax of the three macroalgal species when freshly collected, acclimatised for 2 weeks and when exposed to temperature and light treatments for 1 week, compared to control (Mean ± standard 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).

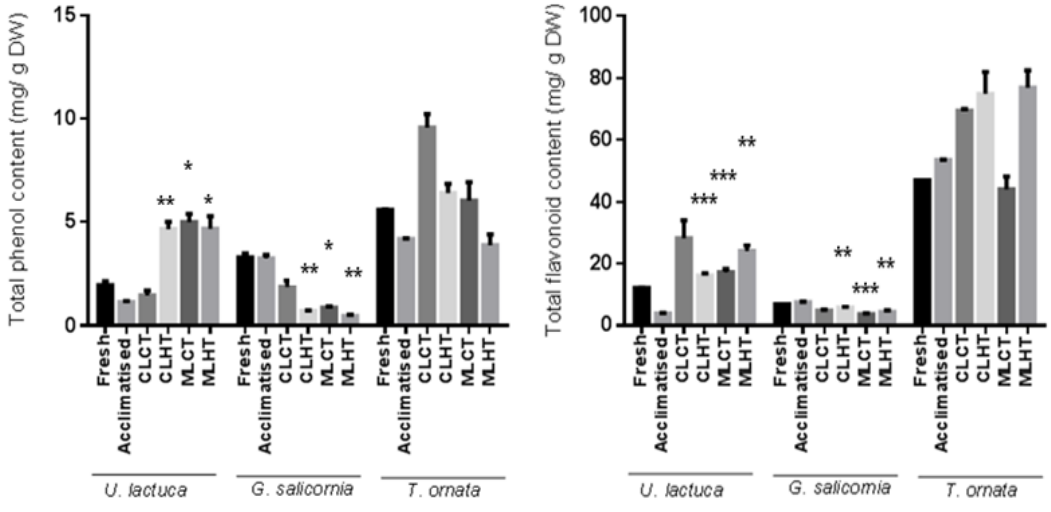

Figure 6. Phytochemical contents. A. Total phenol contents (mg Gallic acid equivalent/g dry weight), B. Total and flavonoid contents (mg Quercetin equivalent/ g dry weight) of the three species of macroalgae when freshly collected, when acclimatized for 2 weeks, and when exposed to temperature and light 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)

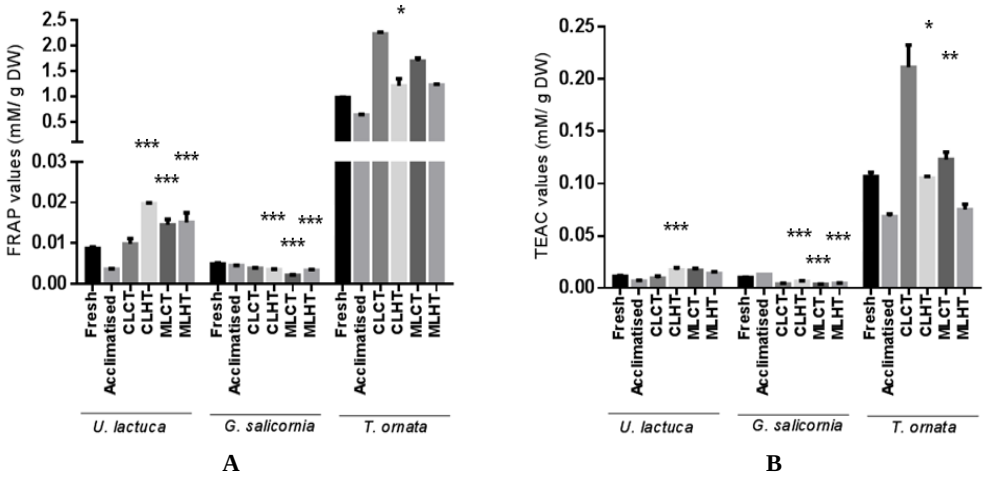

Figure 7. Antioxidant activity. A. Ferric reducing antioxidant potential (FRAP) (mM Ferrous sulfate equivalent/ g dry weight) and B. Trolox Equivalent Antioxidant capacity (TEAC) (mM Trolox equivalent/ g dry weight) values of the three species of macroalgae when freshly collected, when acclimatized for 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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