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Effects of elevated temperatures on growth and photosynthetic performance of polar *Chlorella*

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Abstract Global warming has been the subject of concern in today's world with elevating temperature causing the melting of polar ice and increasing sea level. The aim of this study was to investigate the physiological and photosynthetic performance of two polar *Chlorella*, namely *Chlorella* UMACC 250 and *Chlorella* UMACC 234 to elevating temperatures as might be experienced under future warming scenarios. The cultures were exposed to three different temperatures of 4 ℃, 8 ℃ and 12 ℃. The growth and photosynthetic activity were determined every 2 d for a period of 10 d. At the end of the experiment, the cultures were harvested and analysed for biochemical composition. Both *Chlorella* strains were able to tolerate higher temperatures than their ambient temperature. The final pigments content showed an increasing trend with increased temperatures for both strains. The photosynthetic activities were measured using pulse-amplitude modulation (PAM) fluorometer. The photosynthetic parameters including maximum quantum efficiency (F_v/F_m), maximum relative electron transport rate ($rETR_{max}$), light harvesting efficiency (α) and photoadaptive index (E_k) were derived from the rapid light curves (RLCs). Both *Chlorella* strains showed a slight decline in growth and photosynthetic activities at the initial part of the experiment. However, they showed the ability to recuperate with *Chlorella* UMACC 250 recovers better compared to *Chlorella* UMACC 234. Both *Chlorella* strains showed similar trend in their carbohydrate content at 12 ℃, while the protein content of *Chlorella* UMACC 234 decreased when exposed to increasing temperatures. The results indicated that polar *Chlorella* are able to survive at increased temperatures throughout the experiment.

Keywords **polar, *Chlorella*, photosynthesis, pulse-amplitude modulation (PAM) fluorometry**

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1 Introduction

In recent years, rapid trends of environmental change, mainly increasing temperature has become apparent across the globe (Beardall and Raven, 2004; Teoh et al., 2010). It has been projected by the Intergovernmental Panel on Climate Change (IPCC) model that the temperature change for the end of 21st century (2081–2100) will most likely exceed 1.5 ℃ (IPCC, 2014). The Antarctic freshwater systems consist of benthic microbial communities including green algae and cyanobacteria (Larsen et al., 2014). Increasing temperature in the polar region is a known cause of melting of the sea ice that leads to a rise in sea levels. This phenomenon is detrimental for the Arctic and Antarctic environments, greatly affecting the ecosystem (Larsen et al.,

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2014; Jordan, 2019).

Microalgae consists of both prokaryotic and eukaryotic organisms that rapidly grow in aquatic environment including freshwater, marine water and interestingly, waste water environment (Ravindran et al., 2016). They are unicellular organisms similar to higher plants, capable to undergo photosynthesis to convert light energy to chemical energy. Microalgae is said to be contributing to approximately half of the global primary productivity since they have a more rapid growth and turnover rates compared to higher plants (Gao et al., 2012). Besides being the primary producer of the aquatic ecosystem, microalgae are recognised as living-cell factories. Their biochemical composition such as proteins, carbohydrates and lipids are important for the production of biofuels, and wastewater treatment (Li et al., 2007; Priyadarshani and Rath, 2012). Interestingly, microalgae are also extensively used for feedstock and various food supplements that are beneficial for human's health (Chu, 2012; Kokou et al., 2012; Chew et al., 2017).

The growth of microalgae is regulated by many abiotic factors such as temperature, pH and light intensity (Mendes et al., 2012). Changing environment temperature will initiate changes in microalgae's physiological, chemical and molecular activities (Falkowski and Oliver, 2007; Li et al., 2011). This alterations and changes are a necessary response for them in an attempt of long-term survival. This process is known as acclimation (Valledor et al., 2013). The temperature that ranges between optimal and lethal is varied among different species. Some species might have a narrow range indicating their sensitivity to temperatures, while other species have wider range of temperatures that indicates they are able to survive by adaptation or acclimation (Ras et al., 2013).

Chlorella sp. are known to have optimum growth rates over a broad range of temperatures (Ras et al., 2013; Teoh et al., 2013) and has been highlighted as non-fastidious since it is able to colonize different types of natural environments successfully. A study by Kessler (1985) reported that the optimum growth rates of 17 different tropical *Chlorella* strains were between 26 ℃ and 36 ℃. However, the polar microalgae, *Chlorella saccharophila* was able to grow in temperature range of 5 ℃ to 15 ℃ for over two weeks (Vona et al., 2004). The microalgae *Chlorella* sp. has also been used traditionally by the Japanese as a food supplement as it contains a remarkably high protein content (41%–58%) along with some other species of microalgae such as *Spirulina maxima* and *Synechoccus* sp. (de Morais et al., 2015; Ravindran et al., 2016).

Studies have shown that the protein content decrease in some microalgae when exposed to elevated temperature (Borbély et al., 1985; Teoh et al., 2004; de Castro Araujo and Garcia, 2005; Carvalho et al., 2009). Carbohydrates are regard as microalgae's cellular fuel and possess a vital function as structural support of the membranes. The decrease in this compound can result in affected growth and metabolism of microalgae's cells (de Castro Araujo and Garcia, 2005). Interestingly, there are a few contradicting results reported associated with carbohydrates concentration in microalgae. Increasing temperature normally leads to increasing of carbohydrates contents of some microalgae (Ogbonda et al., 2007) but some microalgae has higher carbohydrates content at lower temperature (Teoh et al., 2004; de Castro Araujo and Garcia, 2005).

Photosynthetic activities of microalgae can be measured using a pulse amplitude modulated (PAM) fluorometer. PAM fluorometry is a frequently used method in the determination of photosynthetic activities in various marine life including macro and microalgae in both the laboratory and *in situ* (Beer and Axelsson, 2004). This technique has been used to measure a wide range of processes associated with photosynthesis where the wellbeing of the algae studied can be assessed since photosynthesis is crucial in photosynthesizing organisms (Sjollema et al., 2014). PAM fluorometry is a non-invasive and non-destructive as well as rapid method that is able to provide direct and immediate information on the photosynthetic activity of the algae compared to the growth inhibition test that requires at least 72 h to produce results. With this more convenient technique, photosynthetic light response curves are easier to be obtained (Ritchie, 2008).

The world is facing global warming at an alarming rate. Water bodies experienced warming at a faster rate than air temperature where ice cover has been depleting, suggesting enhanced radiative warming (Larsen et al., 2014). Anthropogenic emissions since the beginning of Industrial Revolution have led to 1 ℃ of global warming. It is reported that the probability of the warming to reach 1.5 ℃ between the year 2030 and 2052 is high if these emissions persist (IPCC, 2014; Guilyardi et al., 2018). The weather events and the extreme climate change has been of major concern since they have led to disruptions to modern and past societies (Coumou and Rahmstorf, 2012; Cook et al., 2014). However, global warming does not happen overnight. The warming of the earth was observed since the mid-20th century (IPCC, 2011) and recent climate changes are starting to show effects on many natural and human systems (IPCC, 2007). Thus, it is important for us to fully understand the effects of elevated temperatures on organisms, particularly on microalgae. It is beneficial to have the information regarding the physiological response of microalgae to increasing temperature to predict future distributions of microalgae. This knowledge can aid in the findings of the mechanism of survival of microalgae, therefore can be used in future generation of biofuel generation.

The aim of this study was to investigate the response of two *Chlorella* strains originating from the Antarctic to elevated temperatures. The growth, photosynthetic activities, pigments and biochemical composition in these microalgae were determined.

2 Materials and methods

2.1 Algal cultures

The microalgae used in this study, namely *Chlorella* UMACC 250 and *Chlorella* UMACC 234 were obtained from the University of Malaya Algae Culture Collection (UMACC) (Table 1). The *Chlorella* UMACC 250 was isolated from samples collected around Marion Island, Sub-Antarctic (Chu et al., 2002), while UMACC 234 was collected from snow originated from Beal Island, Antarctic. The cultures were maintained in a controlled-environment incubator at 4 ℃, illuminated with cool white fluorescent lamps (42 μmol·m^{-2}·s^{-1}) on 12 h∶12 h light-dark cycle. All cultures were grown in Bold's Basal Medium (BBM) (Nichols and Bold, 1965).

2.2 Experimental design

The microalgal cultures were grown in 2 L conical flasks and placed in light- and temperature-controlled incubators set at different temperatures of 4 ℃ (ambient temperature), 8 ℃ (projected future warming scenarios) and 12 ℃ (projected extreme future warming scenarios). Illumination was provided by cool fluorescent lamps (42 μmol·m^{-2}·s^{-1}) on 12 h∶12 h light-dark cycle. The experiment was carried out in triplicate with the total volume of 1600 mL of each flask. The inoculum (10%) used was from exponential phase cultures standardised at an optical density at 750 nm ($OD_{750\ nm}$) of 0.2.

Table 1 Details of the microalgae selected for this study

Strain name	UMACC number	Habitat *	Origin	Medium #
Chlorella sp.	UMACC 234	FW	Snow from Beal Island, Antarctic	BBM
Chlorella sp.	UMACC 250	FW	Marion Island, Sub-Antarctic	BBM

Notes: *FW: Fresh water; # BBM: Bold's Basal Medium (Nichols and Bold, 1965).

Growth was monitored every 2 d based on at least two parameters: $OD_{750\ nm}$ and cell number or $OD_{750\ nm}$ and chlorophyll-*a* (chl-*a*). Chl-*a* was determined by spectrophotometry after extraction of the filtered samples using glass-fibre filters, 0.47 μm in acetone (Strickland and Parsons, 1968). Chl-*a* was determined using the following formula:

$$\text{Chl-}a\ (\text{mg}\cdot\text{m}^{-3}) : (C_a \times V_a)/V_c ,$$

where, $C_a = 11.6\ (OD_{665\ nm}) - 1.31\ (OD_{645\ nm}) - 0.14\ (OD_{630\ nm})$,

V_a= Volume of acetone (mL) used for extraction,

V_c= Volume of culture (L).

Carotenoid content was determined using the following formula:

$$\text{Carotenoid}\ (\mu\text{g}\cdot\text{mL}^{-1}) : (OD_{452\ nm} \times 3.86 \times V_a) / (V_c) ,$$

where, V_a= Volume of acetone (mL) used for extraction,

V_c= Volume of culture (mL).

As $OD_{750\ nm}$ was measured for all samples, specific growth rate (μ, d^{-1}) based on this parameter was calculated using the following formula: $\mu\ (d^{-1}) = (\text{Ln}N_2 - \text{Ln}N_1)/(t_2 - t_1)$ where N_2 is $OD_{750\ nm}$ at t_2, N_1 is $OD_{750\ nm}$ at t_1, and t_2 and t_1 are times within the exponential phase (Guillard, 1973). Photosynthetic activity was also determined every 2 d using pulse-amplitude modulation (PAM) fluorometer. The samples were harvested at the end of the experiment (stationary phase, day 10) by filtration and used for determination of dry weight and biochemical analysis (protein and carbohydrate).

2.3 Dry weight determination

Blank glass-fibre filters (Fioroni, 0.47 μm) were dried in a forced-air oven at 100 ℃ for 24 h and weighed. A known volume of the algal culture was filtered on a pre-weighed filter and was let dry for 24 h at 100 ℃. The algal dry weight (DW) was determined using the following equation (APHA, 1989):

DW (mg·L^{-1}) = ([Weight of filter with algae (mg)]–[Weight of blank filter (mg)])/[Volume of algal culture (L)]

2.4 Biochemical analysis

The protein concentration of cells was determined using the dye-binding method after extraction in 0.5 mol·L^{-1} NaOH (Bradford, 1976). Carbohydrates contents were extracted in 2 mol·L^{-1} HCl and the concentration were determined using the phenol-sulphuric acid method (Kochert et al., 1978).

2.5 Photosynthetic activity

The photosynthetic activities of microalgae were measured every 2 d using a Diving-PAM fluorometer (Walz, Germany) (McMinn et al., 2010; Keng et al., 2013). Rapid light curve (RLC) was obtained using software control (Wincontrol, Walz). All cultures were dark-adapted for 15 min prior to exposing them to different light level. The maximum quantum efficiency (F_v/F_m) was used to indicate the physiological condition of phytoplankton where the health status of the cells can be estimated. The equation used: $F_v/F_m = (F_m - F_0)/F_m$ where F_m is the maximum fluorescence and F_0 is the minimum fluorescence resulting in the variable fluorescence F_v. The maximum photosynthetic efficiency (α) was determined from the initial slope of the RLC. The quantum yield and irradiance obtained from the end of each light interval was used to calculate the relative electron transport rate (*rETR*). The RLC is a condition where it consists of eight consecutive ten-second intervals of actinic light with increasing intensity. The photoadaptive index (E_k) was obtained from the curve fitting model (Platt

et al., 1980). E_k is the interception point of the α value with the maximum relative electron transport rate ($rETR_{max}$) where $E_k = rETR_{max}/\alpha$.

2.6 Data analysis

The data such as specific growth rate, photosynthetic activities and biochemical composition were analysed by one-way ANOVA followed by comparison of means using Neuman-Keuls Test (Statistica software, Version 5). The differences were considered significant at $p < 0.05$.

3 Results

3.1 Growth trends

Based on specific growth rate measurement (μ, d^{-1}), *Chlorella* UMACC 234 grew best at its ambient temperature (0.35 ± 0.02 d^{-1}), and specific growth rate decreased dramatically with further increase in temperature (Figure 1a) ($p < 0.05$). Meanwhile, the growth of *Chlorella* UMACC 250 was at the highest temperature exposure of 12 ℃ (0.24 ± 0.01 d^{-1}).

Both *Chlorella* sp. from the polar region showed similar trends in their chl-*a* content (Figure 1b). There was a marked increase of chl-*a* content when the cultures were exposed to increasing temperatures, 459.20 ± 0.01 $mg \cdot m^{-3}$ for UMACC 250 and 748.76 ± 0.03 $mg \cdot m^{-3}$ for UMACC 234 at 12 ℃. Same trend was observed in their carotenoid concentration (Figure 1c) where both strains has the highest reading at the highest temperature exposure 0.23 ± 0.01 $\mu g \cdot mL^{-1}$ for *Chlorella* UMACC 250 and 0.41 ± 0.03 $\mu g \cdot mL^{-1}$ for *Chlorella* UMACC 234.

Of the two Antarctic microalgae, the *Chlorella* UMACC 250 strain appeared to be more tolerant to temperature stress while *Chlorella* UMACC 234 was sensitive to temperatures higher than their ambient. In general, polar microalgae are able to survive at a higher temperature range from their ambient temperature.

3.2 Biochemical content

Chlorella UMACC 250 showed an increased value of carbohydrates at 12 ℃ (24.37% ± 5.15%, DW) after a marked decrease at 8 ℃ (11.84% ± 4.12%, DW). Similar finding was observed in *Chlorella* UMACC 234 where the value of carbohydrates increased at 12 ℃ (19.55% ± 6.70%, DW). Carbohydrate was produced more at highest temperature (Figure 2a). The opposite result was obtained for the protein content (Figure 2b) which *Chlorella* UMACC 234 shows a dramatic decrease ($p < 0.05$) of protein content when temperature reaches 12 ℃ (16.08% ± 1.64% DW). However, *Chlorella* UMACC 250 shows increased protein content at 12 ℃ (19.4%, DW) although experiencing a noted decrease to 13.6% DW at 8 °C from 29.2% DW at 4 ℃. Based on the data, more protein was produced by *Chlorella* UMACC 250 in order to survive in elevated temperature.

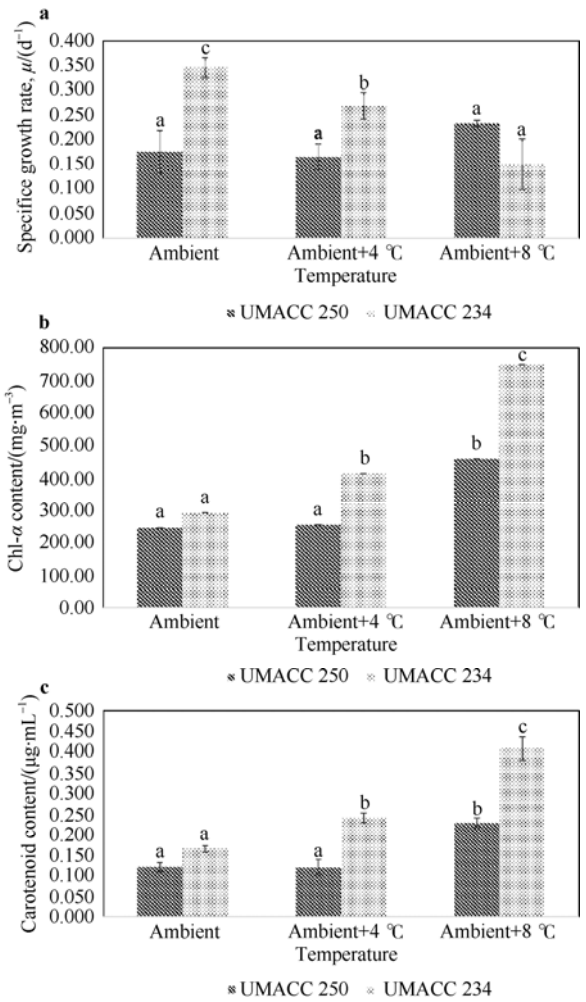

Figure 1 Specific growth rates (**a**) based on $OD_{750\ nm}$, chl-*a* content (**b**) and carotenoid content (**c**) of UMACC 250 and UMACC 234. Vertical bars denote standard deviations from triplicate samples. Different alphabets above the bar charts indicate significant differences ($p < 0.05$) between temperatures for each species.

3.3 Photosynthetic activity

The maximum quantum efficiency (F_v/F_m) (Figure 3a) of *Chlorella* UMACC 250 showed consistent reading from its ambient temperature to the highest temperature exposure with a significant increase ($p < 0.05$) from 0.55 ± 0.02 at 4 °C to 0.60 ± 0.01 at 12 °C. *Chlorella* UMACC 234 shows a significant increase ($p < 0.05$) from 0.37 ± 0.04 at its ambient temperature to 0.47 ± 0.01 at 8 °C after 10 d of exposure. The F_v/F_m indicates the health of the microalgae being studied and *Chlorella* UMACC 234 showed potentially compromised health when temperature reached 12 °C. The light harvesting efficiency (α) (Figure 3b) for *Chlorella* UMACC 250 were reported highest at the highest temperature (0.40 ± 0.05). *Chlorella* UMACC 234 shows similar trend with α the highest at 12 ℃ (0.26 ± 0.00). The

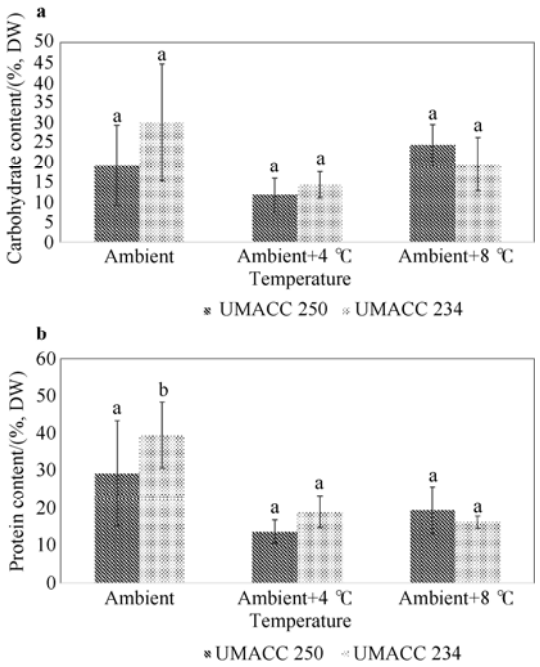

Figure 2 Carbohydrate content (**a**) and protein content (**b**) of UMACC 250 and UMACC 234. Vertical bars denote standard deviations from triplicate samples. Different alphabets above the bar charts indicate significant differences ($p < 0.05$) between temperatures for each species.

maximum relative electron transport rate, $rETR_{max}$ (μmol·m^{-2}·s^{-1}, electrons) (Figure 3c) for *Chlorella* UMACC 250 showed a decreasing trend as temperature increased. In contrast, *Chlorella* UMACC 234 shows high $rETR_{max}$ reading at highest temperature (276.67 ± 90.05 μmol·m^{-2}·s^{-1}, electrons). There was no consistent trend for the photoadaptive index, E_k (Figure 3d) of both strains where *Chlorella* UMACC 250 shows consistent decreased in E_k reading as temperature elevated while *Chlorella* UMACC 234 has highest E_k reading at 8 °C (1273.38 ± 492.93 μmol·m^{-2}·s^{-1}, photons) and started to decrease as temperature reached 12 °C (1096.06 ± 450.51 μmol·m^{-2}·s^{-1}, photons).

4 Discussion

Based on the results, both polar *Chlorella* UMACC 250 and *Chlorella* UMACC 234 strains have distinct growth trends in accordance to temperature (Figure 1). It can be observed that the polar UMACC 250 is eurythermal where it can tolerate a wider range of temperature (Lee et al., 2018) from 4 ℃ to 12 ℃ and can adapt a temperature higher than its ambient temperature, it has the highest specific growth rate (μ) at highest temperature exposure. This proves that polar microalgae are psychrotrophic since they are cold-tolerant microorganism that possesses the ability to grow at low temperatures but have optimal growth temperature at above 20 ℃ (Shukla et al., 2011). Similar finding was reported by

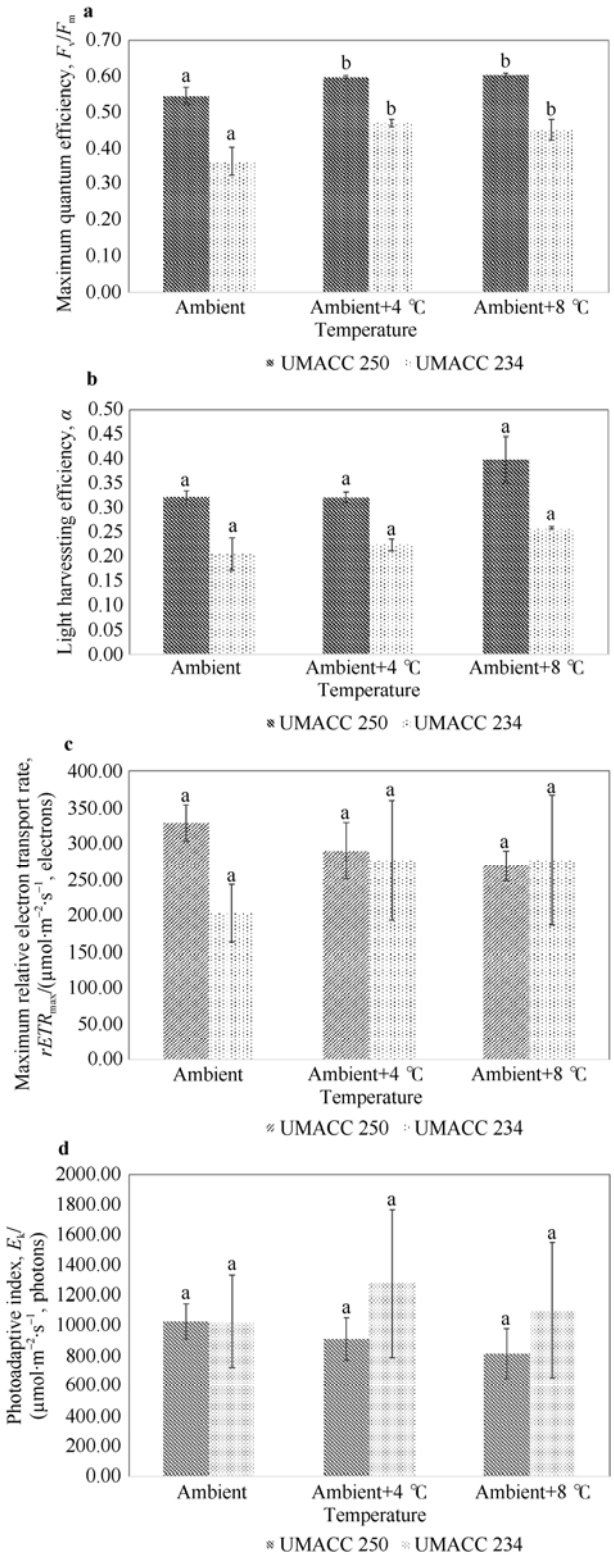

Figure 3 **a**, Maximum quantum efficiency (F_v/F_m); **b**, Light harvesting efficiency (α); **c**, Maximum relative electron transport rate, $rETR_{max}$; **d**, Photoadaptive index, E_k of both UMACC 250 and UMACC 234. Vertical bars denote standard deviations from triplicate samples. Different alphabets above the bar charts indicate significant differences ($p < 0.05$) between temperatures for each species.

Teoh et al. (2004) where the polar microalgae were able to survive even at 30 ℃. In current study, the findings indicated that *Chlorella* UMACC 234 strain is more sensitive to temperature elevation when compared to *Chlorella* UMACC 250 since its specific growth rate (μ) decreases markedly as temperature increases. It can be said that *Chlorella* UMACC 234 has reached its optimum growth temperature close to its ambient temperature. At temperature below the optimum level, growth will increase with increasing temperature but will decrease significantly at temperature above the optimum level (Teoh et al., 2010). Temperature elevations are commonly link to increasing growth of microalgae as proven in a study by Shukla et al. (2013) where increased temperature of up to 23 ℃ stimulated the growth of polar *Chlorella mirabilis*.

Both *Chlorella* strains show increasing trend of chl-*a* and carotenoid content as temperature increases. Past studies have reported that adaptation to low temperature imitates the acclimation to condition of high irradiance (El-Sabaawi and Harrison, 2006), thus lower chlorophyll content is considered as a microalgae's normal response to high irradiance. An increased in pigment composition under high temperature indicates that higher energy is supplied by light harvesting process (Geider et al., 1997). Furthermore, pigment synthesis might speed up at elevated temperature (Cao et al., 2016). However, different environmental conditions exposures affect chlorophyll contents in a species-specific manner (da Silva Ferreira and Sant'Anna, 2017). High temperature can lead to stressful condition upon microalgae that eventually speeds up the production of excess free radicals. In order to mitigate the harmful effects of these radicals, algae cells will produce molecules with anti-oxidant properties, hence the increasing content of carotenoids (Ras et al., 2013).

In general, the biochemical content for each polar strain varies. Carbohydrate content for both *Chlorella* strains is abundant at highest temperature exposure with *Chlorella* UMACC 250 being the highest. However, inconsistency of carbohydrates accumulation with temperature have been observed in previous studies. For example, a study conducted on the microalgae *Pavlova lutheri* shows that carbohydrate content increases at temperature of 18 ℃, and slightly decrease afterwards at higher temperature of 22 ℃ (Carvalho et al., 2009).

In current study, protein content in both strains are much lower in increasing temperature compared to their ambient temperature with *Chlorella* UMACC 234 shows a consistent dramatic decrease as temperature elevates. This result is similar to the findings where polar *Chlorella* accumulated protein and lipids under lower temperatures and as temperature elevates; its metabolism tends to synthesis soluble sugar (Cao et al., 2016). Typically, the biochemical content of a fast-growing photosynthetic microalgal cell is generally associated with a low carbohydrate and high protein content. However, under environmental stress conditions where cells might have reached their stationary growth phase, more photoassimilated carbon is link to carbohydrates or lipids (Zhu et al., 1997). In spite of that, there is no consistent trend in biochemical composition in response to temperature. It was found to be species specific (Teoh et al., 2010).

Under varying environmental conditions, photosynthetic organisms will strive to maintain an equilibrium between energy supply through electron transport and energy utilized via carbon fixation (Maxwell et al., 1994). Photosynthetic performance measured by using fluorescence can be associated with its (relative) electron transport rate and followed by growth rate, thus giving a faster estimates of productivity (Baker, 2008; Malapascua et al., 2014). The reading of F_v/F_m indicates the 'health' of the microalgae being studied where value > 0.5 is considered as healthy. *Chlorella* UMACC 250 shows value of > 0.5 until day 10 for all temperature exposures indicating healthy microalgae. In contrast, F_v/F_m value for *Chlorella* UMACC 234 shows < 0.5 in all temperature exposures suggesting the depletion of nutrient in experimented cultures and possible temperature stress due to the transportation of flasks from one temperature to another.

The α is a function for light absorption efficiency. It is regard as the measure of efficiency of light harvesting activity of microalgae. The α reading for both strains are highest at 12 ℃. As temperature increases, the demand for available carbon will also increases, as well as a possible enhancement of RuBisCo activity (Fu et al., 2007). Therefore, both *Chlorella* strains were able to change to greater light-harvesting efficiency. *Chlorella* UMACC 250 has the highest value of 0.4 at 12 ℃, suggesting that it harvests light more efficiently compared to UMACC 234. This can be seen as one of the reasons why *Chlorella* UMACC 250 shows a higher μ at elevated temperature.

The *rETR* is an estimation of the electron flow rate through the photosynthetic chain (Malapascua et al., 2014; Gomes et al., 2017). Interestingly, despite being the more tolerant strain, *Chlorella* UMACC 250 showed significant decrease in $rETR_{max}$ from values above 300 $m^{-2}\cdot s^{-1}$ at 4 ℃ to values below 300 $m^{-2}\cdot s^{-1}$ at 12 ℃. It has been concluded that strains originated from bright environments tend to have higher values than those that grow in low light environment. The same trend was observed in the photoadaptive index, E_k where *Chlorella* UMACC 250 had decreasing value as temperature increased. The finding was explained in a study where the rise in α during exposure to increased temperature led to a lower light level for saturation of E_k. This shows that the efficiency of light harvesting had increased (Fu et al., 2007).

In conclusion, both polar *Chlorella* strains studied are able to grow at temperature higher than their ambient temperature. They tend to decrease their protein content and increase their carbohydrates content in order to adapt to elevated culture temperatures. Further research involving

proteomic studies to investigate the types of proteins expressed during the period of high temperature exposure of the polar microalgae will be carried out.

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References

American Public Health Association (APHA). 1989. Standard methods for the examination of water and wastewater, 17th edn. American Public Health Association, Washington. DC.

Baker N R. 2008. Chlorophyll fluoresence: A probe of photosynthesis *in vivo*. Annu Rev Plant Biol, 59: 89-113.

Beardall J, Raven J A. 2004. The potential effects of global climate change on microalgal photosynthesis growth and ecology. Phycologia, 43(1): 26-40.

Beer S, Axelsson L. 2004. Limitations in the use of PAM fluorometry for measuring photosynthetic rates of macroalgae at high irradiances. Eur J Phycol, 39(1): 1-7.

Borbély G, Surányi G, Korcz A, et al. 1985. Effect of heat shock on protein synthesis in the cyanobacterium *Synechococcus* sp. strain PCC 6301. J Bacteriol, 161(3): 1125-1130.

Bradford M M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem, 72: 248-254.

Cao K, He M, Yang W, et al. 2016. The eurythermal adaptivity and temperature tolerance of a newly isolated psychrotolerant Arctic *Chlorella* sp. J Appl Phycol, 28(2): 877-888.

Carvalho A P, Monteiro C M, Malcata F X. 2009. Simultaneous effect of irradiance and temperature on biochemical composition of the microalga *Pavlova lutheri*. J Appl Phycol, 21(5): 543-552.

Chew K W, Yap J Y, Show P L, et al. 2017. Microalgae biorefinery: High value products perspectives. Bioresour Technol, 229: 53-62.

Chu W L. 2012. Biotechnological applications of microalgae. IeJSME, 6(Suppl 1): S24-S37.

Chu W L, Yuen Y Y, Wong C Y, et al. 2002. Isolation and culture of microalgae from the Windmill Islands region, Antarctica. Proceedings of the Malaysian International Seminar on Antarctica: Opportunities for Research, 5-6 August 2002. Kuala Lumpur, 53-59.

Cook B I, Smerdon J E, Seager R, et al. 2014. Global warming and 21st century dying. Clim dynam, 43(9-10): 2607-2627.

Coumou D, Rahmstorf S. 2012. A decade of weather extremes. Nat Clim Change, 2(7): 491-496.

da Silva Ferreira V, Sant'Anna C. 2017. Impact of culture conditions on the chlorophyll content of microalgae for biotechnological applications. World J Microbiol Biotechnol, 33: 20, doi: 10.1007/s11274-016-2181-6.

de Castro Araújo S, Garcia V M T. 2005. Growth and biochemical composition of the diatom *Chaetoceros* cf. *wighamii* brightwell under different temperature, salinity and carbon dioxide levels. I. Protein, carbohydrates and lipids. Aquaculture, 246(1-4): 405-412.

de Morais M G, Vaz B D S, de Morais E G, et al. 2015. Biologically actives metabolites synthesized by microalgae. BioMed Res Int, 4: 835761, doi: 10.1155/2015/835761.

El-Sabaawi R, Harrison P J. 2006. Interactive effects of irradiance and temperature on the photosynthetic physiology of the pennate diatom *Pseudo-nitzschia granii* (Bacillario-phyceae) from the Northeast Subarctic Pacific. J Phycol, 42(4): 778-785.

Falkowski P G, Oliver M J. 2007. Mix and match: how climate selects phytoplankton. Nat Rev Microbiol, 5: 813-819.

Fu F X, Warner M E, Zhang Y, et al. 2007. Effects of increased temperature and CO_2 on photosynthesis, growth, and elemental ratios in marine *Synechococcus* and *Prochlorococcus* (cyanobacteria). J Phycol, 43: 485-496.

Gao K, Helbling E W, Häder D P, et al. 2012. Responses of marine primary producers to interactions between ocean acidification, solar radiation, and warming. Mar Ecol Prog Ser, 470: 167-189.

Geider R J, Macintyre H L, Kana T M. 1997. Dynamic model of phytoplankton growth and acclimation: responses of the balanced growth rate and the chlorophyll *a*: carbon ratio to light, nutrient-limitation and temperature. Mar Ecol Prog Ser, 148: 187-200.

Gomes T, Xie L, Brede D, et al. 2017. Sensitivity of the green algae *Chlamydomonas reinhardtii* to gamma radiation: photosynthetic performance and ROS formation. Aquat Toxicol, 183: 1-10.

Guilayrdi E, Lescarmontier L, Matthews R, et al. 2018. IPCC special report "Global warming of 1.5 °C". Summary for teachers.

Guillard R R L. 1973. Division rates//Stein J R. Handbook of phycological methods: Volume 1, Cambridge: University Press, 289-312.

Intergovernmental Panel on Climate Change (IPCC). 2007. Change, on Climate. http://streitcouncil.org/uploads/PDF/Report-ClimateChange 2007ImpactsAdaptationandVulnerability.pdf.

Intergovernmental Panel on Climate Change (IPCC). 2011. Summary for Policymakers. In: IPCC Special Report on Renewable Energy Sources and Climate Change Mitigation. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Intergovernmental Panel on Climate Change (IPCC). 2014. Climate Change 2014 Synthesis Report Summary for Policymakers. https://www.ipcc.ch/pdf/assessmentreport/ar5/syr/AR5_SYR_FINAL_SPM.pdf.

Jordan L. 2019. Global warming in the polar regions: The effects of climate change. https://www.gapyear.com/articles/features/global-warming-in-the-polar-regions.

Keng F S L, Phang S M, Rahman N A R, et al. 2013. Volatile halocarbon emissions by three tropical brown seaweeds under different irradiances. J Appl Phycol, 25(5): 1377-1386.

Kessler E. 1985. Upper limits of temperature for growth in *Chlorella*. Plant Syst Evol, 151(1): 67-71.

Kochert G, Hellebust J A, Craigie J S. 1978. Carbohydrate determination by the phenol sulfuric acid method//Hellebust J A, Craigie J S. Handbook of phycological methods: Physiological and biochemical methods, Cambridge: Cambridge University Press, London, UK, 95-97.

Kokou F, Makridis P, Kentouri M, et al. 2012. Antibacterial activity in

microalgae cultures. Aquac Res, 43: 1520-1527.

Larsen J N, Anisimov O A, Constable A, et al. 2014. Climate change 2014: Impacts, adaptations and vulnerability. Polar Regions, Cambridge University Press, Cambridge and New York, 1567-1612.

Lee K K, Lim P E, Poong S W, et al. 2018. Growth and photosynthesis of *Chlorella* strains from polar, temperate and tropical freshwater environments under temperature stress. J Ocean Limnol, 36(4): 1266-1279.

Li X, Hu H Y, Zhang Y P. 2011. Growth and lipid accumulation properties of a freshwater microalga *Scenedesmus* sp. under different cultivation temperature. Bioresour Technol, 102: 3098-3102.

Malapascua J R F, Jerez C G, Sergejevová M, et al. 2014. Photosynthesis monitoring to optimize growth of microalgal mass cultures: application of chlorophyll fluorescence techniques. Aquat Biol, 22: 123-140.

Maxwell D P, Falk S, Trick C G, et al. 1994. Growth at low temperature mimics high-light acclimation in *Chlorella vulgaris*. Plant Physiol, 105: 535-543.

McMinn A, Martin A, Ryan K. 2010. Phytoplankton and sea ice algal biomass and physiology during the transition between winter and spring. Polar Biol, 33: 1547-1556.

Mendes L F, Vale L A S, Martins A P, et al. 2012. Influence of temperature, light and nutrients on the growth rates of microalga *Gracilaria domingensis* in synthetic seawater using experimental design. J Appl Phycol, 24: 1419-1426.

Nichols H W, Bold H C. 1965. *Trichosarcina polymorpha gen.* et sp. Nov. J Phycol, 1: 34-38.

Ogbonda K H, Aminigo R E, Abu G O. 2007. Influence of temperature and pH on biomass production and protein biosynthesis in a putative *Spirulina* sp. Bioresour Technol, 98(11): 2207-2211.

Priyadarshani I, Rath B. 2012. Commercial and industrial application of microalgae – A review. Journal of Algal Biomass Utilization, 4: 89-100.

Ras M, Steyer J P, Bernard O. 2013. Temperature effect on microalgae: a crucial factor for outdoor production. Rev Environ Sci Bio/Technol, 12(2): 153-164.

Ravindran B, Gupta S K, Cho W M, et al. 2016. Microalgae potential and multiple roles – current progress and future prospects – an overview. Sustainability, 8: 1-16.

Ritchie R J. 2008. Fitting light saturation curves measured using modulated fluorometry. Photosynth Res, 96: 201-215.

Shukla S P, Kvíderová J, Elster J. 2011. Nutrients requirement of polar *Chlorella*-like species. Czech Polar Reports, 1(1): 1-10.

Shukla S P, Kvíderová J, Triska J, et al. 2013. *Chlorella mirabilis* as a potential species for biomass production in low-temperature environment. Front Microbiol, 4: 97.

Sjollema S B, Van Beusekom S A M, Van Der Geest H G, et al. 2014. Laboratory algal bioassays using PAM fluorometry: effects of test conditions on the determination of herbicide and field sample toxicity. Environ Toxicol Chem, 33(5): 1017-1022.

Strickland J D H, Parsons T R. 1968. A practical handbook of seawater analysis. Fish Res Board Can Bull, 167: 311.

Teoh M L, Chu W L, Marchant H, et al. 2004. Influence of culture temperature on the growth, biochemical composition and fatty acid profiles of six Antarctic microalgae. J Appl Phycol, 16(6): 421-430.

Teoh M L, Phang S M, Chu W L. 2010. Effect of temperature change on physiology and biochemistry of algae: A review. Malays J Sci, 29(2): 82-97.

Teoh M L, Phang S M, Chu W L. 2013. Response of Antarctic, temperate, and tropical microalgae to temperature stress. J Appl Phycol, 25: 285-297.

Valledor L, Furuhashi T, Hanak A M, et al. 2013. Systemic cold stress adaptation of *Chlamydomonas reinhardtii*. Mol Cell Proteomics, 12(8): 2032-2047.

Vona V, Di Martino Rigano V, Lobosco O, et al. 2004. Temperature responses of growth, photosynthesis, respiration and NADH: nitrate reductase in cryophilic and mesophilic algae. New Phytol, 163(2): 325-331.

Zhu C J, Lee Y K, Chao T M. 1997. Effects of temperature and growth phase on lipid and biochemical composition of *Isochrysis galbana* TK1. J Appl Phycol, 9: 451-457.