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Solar cultivation of microalgae in a desert environment for the development of techno-functional feed ingredients for aquaculture in Qatar

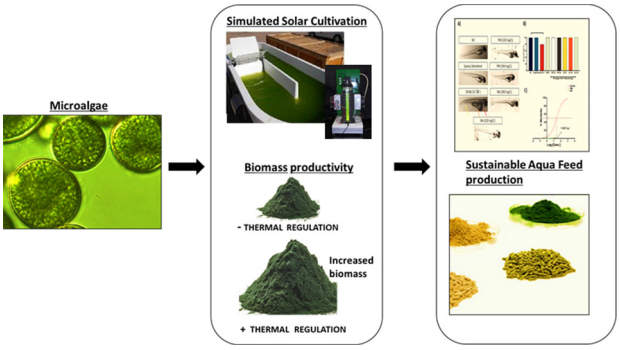
Rihab Rasheed [a,*], Mahmoud Thaher [a], Nadin Younes [c,f], Touria Bounnit [a], Kira Schipper [a], Gheyath K. Nasrallah [c,f], Hareb Al Jabri [a,b], Imma Gifuni [d], Olivier Goncalves [e], Jeremy Pruvost [e]

[a] Center for Sustainable Development, College of Arts and Sciences, Qatar University, Doha 2713, Qatar
[b] Department of Biological and Environmental Sciences, College of Arts and Sciences, Qatar University, Doha 2713, Qatar
[c] Biomedical Research Center, Qatar University, Doha 2713, Qatar
[d] Algosource Technologies, 7, Rue Eugène Cornet, 44600 Saint-Nazaire, France
[e] CNRS, GEPEA, UMR 6144, Université de Nantes, Oniris, F-44600 Saint-Nazaire, France
[f] Department of Biomedical Sciences, College of Health Sciences, Member of QU Health, Qatar University, Doha 2713, Qatar

HIGHLIGHTS

- Two microalgae studied for annual productivity in Qatar, and fish-feed suitability.
- Biomass productivities of up to 32.9 g/m^2/d found under Qatari summer conditions.
- Simulated thermal regulation increased annual biomass productivities.
- Up to 54% proteins and presence of essential PUFAs make biomass suitable for feed.
- Zero toxicity on zebrafish embryos even at highest concentrations

GRAPHICAL ABSTRACT



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ABSTRACT

The demand for aquaculture feed will increase in the coming years in order to ensure food security for a growing global population. Microalgae represent a potential fish-feed ingredient; however, the feasibility of their sustainable production has great influence on its successful application. Geographical locations offering high light and temperature, such as Qatar, are ideal to cultivate microalgae with high productivities. For that, the environmental and biological interactions, including field and laboratory optimization, for solar production and application of two native microalgae, *Picochlorum maculatum* and *Nannochloris atomus*, were investigated as potential aquaculture feed ingredients. After validating pilot-scale outdoor cultivation, both strains were further investigated under simulated seasonal conditions using a thermal model to predict light and culture temperature cycles for the major climatic seasons in Qatar. Applied thermal and light variations ranged from 36 °C and 2049 μmol/m^2/s in extreme summer, to as low as 15 °C and 1107 μmol/m^2/s in winter, respectively. Biomass productivities of both strains varied significantly with maximum productivities of 32.9 ± 2.5 g/m^2/d and 17.1 ± 0.8 g/m^2/d found under moderate summer conditions for *P. maculatum* and *N. atomus*, respectively. These productivities were significantly reduced under both extreme summer, as well as winter conditions. To improve annual biomass productivities, the effect of implementation of a simple ground heat exchanger for thermal regulation of raceway ponds was also studied. Biomass productivities increased significantly, during extreme seasons due to respective cooling and heating of the culture. Both strains produced high amounts of proteins during winter, 54.5 ± 0.55% and 44 ± 2.25%, while lipid contents were high during summer reaching up to 29.6 ± 0.75

* Corresponding author.
E-mail address: rihabrasheed@qu.edu.qa (R. Rasheed).

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and 28.65 ± 0.65%, for *P. maculatum* and *N. atomus* respectively. Finally, using acute toxicity assay with zebra fish embryos, both strains showed no toxicity even at the highest concentrations tested, and is considered safe for use as feed ingredient and to the environment.

1. Introduction

Food security is defined as a condition in which all people have access to food that is nutritious and sufficient in quantity. However, with the rapidly increasing human population, food insecurity is becoming a global threat (Kusmayadi et al., 2021). Questions arise whether traditional agriculture, both for crops and livestock, will be able to support the growing needs sustainably. Additionally, for desert environments such as in Qatar, cultivation of food crops is difficult due to non-availability of arable land and lack of fresh water sources. Hence, sustainable solutions, will be necessary to ensure food security for future generations (Mok et al., 2020).

Nowadays, aquaculture plays a very important role in fish production and accounts for over 50% of fish supply for human consumption. This trend will be increasing over the coming years (Dineshbabu et al., 2019). Fish Meal and Fish Oil are the commonly used feed ingredients in aquaculture due to their excellent nutritional properties. However, their production is economically, ethically and environmentally unsustainable in all economies (Bongiorno et al., 2020). A high potential solution is the use of microalgal biomass as a source of functional and nutritional feed ingredient in aquaculture. The photosynthetically driven biomass production process utilizes (sun) light with a high conversion yield achieving greater productivities compared to plants (Vecchi et al., 2020). Additionally, their biomass has high usefulness in serving as resources of proteins, essential amino acids, PUFAs, carbohydrates, carotenoids, vitamins, and minerals (Gullón et al., 2021; Gullón et al., 2020).

Microalgal cultivation mainly depends on availability of sunlight, water resource and CO_2 supply (Özçimen et al., 2018). Their scale up production systems are classified into closed (photobioreactors) and open (raceway ponds) systems. While the closed systems produce high cell densities and productivities due to controlled culture conditions, it is the traditional raceway ponds that are more feasible at industrial scale, accounting for 90% of global microalgae production (Fernández et al., 2021). Here, however, biomass productivity and biochemical composition are influenced by environmental and operational factors, such as light and temperature. For light, over the course of a single day or on a seasonal scale, solar radiations will vary from zero during the night, to potentially over-saturating levels at mid-day (Mazzelli et al., 2020). Furthermore, light availability can even vary within the cultivation systems due to the mixing and shading of dense cultures (Pruvost et al., 2008). Under controlled artificial light conditions such as in photobioreactors, optimal biomass concentrations and then maximal biomass productivities can be achieved easily, however, this is as far more challenging under daily/yearly light cycles for outdoor cultivation (Takache et al., 2010; Pruvost et al., 2011; Cuaresma et al., 2011). Nonetheless, there is a threshold above which microalgae cannot acclimatize to high light intensities, leading to growth inhibition and cell-death (Juneja et al., 2013).

On the other hand, temperature can also modify the physiological processes of the strain. (Van Wagenen et al., 2012; Brindhadevi et al., 2021). Studies have shown that for certain microalgae strains, lipid contents can increase with increasing temperatures, however, this response is strain-dependent (Fakhry and Maghraby, 2015). Strains such as *Tetraselmis subcordiformis* and *Nannochloropsis oculata* showed a decrease of PUFAs and neutral lipids, while *Dunaliella salina* exhibited increased PUFA content at high and low temperatures respectively (Brindhadevi et al., 2021). Nevertheless, temperatures beyond and below optimal levels have detrimental effects on the biomass productivity resulting from inhibited cell multiplication (Sheng et al., 2011).

In recent years, several theoretical models have been developed to investigate the effects of light and temperature on microalgal productivity. However, understanding the interactive effects of vital stressors, such as temperature and light, on the physiological performance of photosynthetic organisms is fundamental to predicting their responses to natural environmental conditions throughout the year. One such example is a thermal model for simulations of the temperature profiles of race-way ponds for microalgal production in Qatar developed by Pruvost et al. (2019). Additionally, this model also can be used to investigate culture strategy optimization methods to improve biomass productivities, resulting from ground heat exchangers implemented to the ponds for heating or cooling the cultures. Qatar is a peninsula in the Arabian Gulf and is characterized by a desert climate with abundant sunlight and high temperatures, which have the potential to either favor or inhibit productive algae biomass cultivation (Das et al., 2016). With two major seasons, summer and winter, the selection of appropriate strains that can withstand the diurnal fluctuations in light and temperature for both seasons is a prerequisite in order to sustain the year-round production of biomass with economic feasibility.

Therefore, in this work, two native strains were evaluated for their annual productivity potential for cultivation in Qatar. To date, only a limited number of studies demonstrated the robustness of strains outdoor, before their evaluation in indoor photo bioreactors (Morillas-España et al., 2021). Therefore, initially the strains were subjected to outdoor cultivation under natural environmental conditions. Further, summer and winter conditions obtained from a validated thermal model enabled the prediction of conditions in open ponds. This conditions were simulated in lab-scale photo bioreactors, and the tolerance of both strains during these diurnal fluctuations in light and temperature was assessed in terms of biomass productivities (Pruvost et al., 2019). In addition, the effect of implementation of a thermal ground heat exchanger was investigated for potential improvement of these productivities in outdoor raceways. The relevance of the microalgae as fish feed ingredient was determined following the variation in their biochemical composition for the different year periods. Finally, evaluating many diets supplemented by different microalgae directly in aquaculture species is a high cost and long-term endeavor. Therefore, new strategies are needed to accelerate experimental dietary supplement processing and to make this task cost-effective (Ulloa et al., 2014). One plausible strategy is to perform preliminary studies in zebrafish, an animal model in which many diets can be assessed short-term and at lower costs than at fish farms (Ulloa et al., 2014; Hedrera et al., 2013). Thus, to verify the market potential of the produced algal biomass, a state-of-the-art toxicity assay on zebra fish embryos was performed to prove their suitability as a sustainable and safe feed ingredient in aquaculture.

2. Materials and methods

2.1. Microalgae strains & cultivation conditions

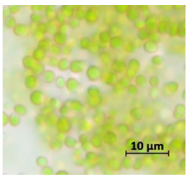
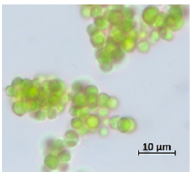
The microalgae strains were isolated from various marine environments around the peninsula of Qatar as defined in Table 1.

Strains were cultured in locally sourced sterilized seawater with a natural salinity of 40 ppt. The water was enriched with F/2 media containing 75 mg/L $NaNO_3$, 5 mg/L $NaH_2PO_4{\cdot}H_2O$, 4.36 mg/L Na_2EDTA, 3.15 mg·L^{-1} $FeCl_3{\cdot}6H_2O$, 0.02 mg/L $MnCl_2{\cdot}4H_2O$, 0.02 mg/L $ZnSO_4{\cdot}7H_2O$, 0.01 mg/L $CoCl_2{\cdot}6H_2O$, 0.01 mg/L $CuSO_4{\cdot}5H_2O$, 0.006 mg/L $Na_2MoO_4{\cdot}2H_2O$, 0.2 mg/L thiamine-HCl, 0.01 mg/L vitamin B12, and 0.1 mg/L biotin, pH adjusted to 8.0. Stock cultures were maintained in 250 mL conical flasks with a working volume of 100 mL in an illuminated Innova 44 Shaker Incubator (New Brunswick Scientific) at 150 RPM, 30 °C, 70 μmol photons·m^{-2}·s^{-1} and 12:12 h light: dark cycle.

2.2. Outdoor cultivation

PM and NA were inoculated in duplicate open raceway culture systems (1 m^2 (2.5 m × 0.4 m), 200 L). The media for the experiment was five times

Table 1
Details of microalgae strains investigated, incl. origin, accession number and code used.

Scientific name	Microscopic observation	Code used	Accession number	Culture collection	Biotope
Picochlorum maculatum		PM	MG149785	Qatar University culture collection cyanobacteria and microalgae (QUCCCM)	Fish pond, Tbeka Qatar
Nannochloris atomus		NA	KM985399	Qatar University culture collection cyanobacteria and microalgae (QUCCCM)	Rocks on Wakra beach

concentrated F2 media (5P5N) to avoid from nutrient limitation. Both strains were cultivated in batch with similar starting densities, in the presence of carbon dioxide, automatically injected based on pH set at 8. Temperature and light were recorded throughout the experiment.

2.3. PBR cultivation and growth optimization of the strains

2.3.1. Photobioreactors

Indoor optimization studies for both strains were performed in conical benchtop photobioreactors (ePBR, 101 Phenometrics, USA). The PBRs were designed to mimic open raceway pond cultivation conditions with light and temperature cycles, projecting LED light source from the top, 18 cm culture depth and a working volume capacity of 410 mL. The illuminated surface area S_{light} was 0.00229 m^2 (i.e. specific illuminated surface of 5.5 m^{-1}). The PBRs were operated in continuous mode, with fully automated medium injection and biomass harvesting at a constant dilution rate (D) of 0.017/h. 100% air mixture was bubbled through the reactors continuously with intermittent mixing of 15% CO_2 enriched air, to maintain the pH at 8 ± 0.5. The culture was agitated using a magnetic stirrer bar set at 450 rpm. The PBRs were inoculated with PM and NA at fixed OD and allowed to grow in batch for 3–4 days followed by continuous mode. Daily measurements of growth (Optical Density, Dry weights, cell count) were taken, until the culture reached a steady state (i.e. stabilized periodic regime over the 24 h cycle).

2.3.2. Thermal cycles

Thermal cycles as encountered in outdoor raceways were generated from a thermal model which was previously validated (Pruvost et al., 2019). The diurnal variation in culture temperature along with light intensities (i.e. photons flux density, PFD) as predicted by the model was used to simulate conditions encountered in raceways for summer and winter periods. Based on the results obtained over the year, averaged summer and winter conditions were determined. Because of the extreme conditions that can be encountered in Qatar during summer, an additional cycle was investigated named hereafter "extreme summer conditions", as given by the largest PFD and temperature cycles obtained from the year prediction.

Once the cells adapted to the regime and reached the steady state, samples were collected for measurement of growth parameters on three different successive days.

Furthermore, additional thermal cycles were applied for all seasons (hereafter named "regulated conditions"), which simulate the effect of thermal regulation of outdoor raceway culture systems through the use of ground heat exchanger. Such a system was described previously and is composed of a simple closed fluid loop with circulation between the culture and the ground having a constant temperature throughout the year (around 28 °C in Qatar). This could potentially cool the culture during summer and heat it during winter seasons. (Pruvost et al., 2019). Based on this approach the profiles applied are explained as below:

i) *Summer*: Temperature fluctuation corresponding to temperature cycle over 24 h, varying from 23.9 °C to 31.5 °C (without regulation) and 26.4 °C to 31.6 °C (with regulation, here after named as reg Summer), combined with light (light/dark −13:11 h), corresponding to light intensity ranging from 0 to 1489.9 µmol/m^2/s (Fig. 1 A).
ii) *Extreme Summer*: Temperature fluctuation corresponding to temperature cycle over 24 h, varying from 27.7 °C to 36.3 °C (without regulation) and 26.2 °C to 31.6 °C (with regulation, here after named as reg Ext Summer), combined with light (light/dark −13:11 h), corresponding to light intensity ranging from 0 to 2049 µmol/m^2/s (Fig. 1 B).
iii) *Winter*: Temperature fluctuation corresponding to temperature cycle over 24 h, varying from 15.1 °C to 21.5 °C (without regulation) and 28.5 °C to 30.9 °C (with regulation, here after named as reg winter), combined with light (light/dark-10:14 h), corresponding to light intensity ranging from 0 to 1107 µmol/m^2/s (Fig. 1 C).

The maximum culture temperature simulated was 36.3 °C during extreme summer and the lowest temperature experienced by the strains was during winter up to 15.13 °C.

2.4. Biomass concentration and areal productivity

15 mL culture sample was filtered on to glass microfiber filters (Whatman GF/C, Sigma-Aldrich, USA) using vacuum pump (Buchner filtration system, Merck Millipore, Darmstadt, Germany). The filter was then washed with 10 mL of 0.5 M ammonium formate to remove salts that may interfere with the final weights. The filters were dried in the oven at 105 °C for 24–48 h.

Volumetric productivity was calculated using:

$$P_x = C_X \cdot D \tag{1}$$

where *Px* is volumetric productivity (g/m^3/d), *Cx* is biomass concentration (g/m^3), and D is the dilution rate (h^{-1}).

Areal productivity S_x in g/m^2/d was obtained by:

$$S_x = \frac{P_x}{a_{light}} \tag{2}$$

where a_{light} is specific illuminated area per culture volume.

Photosynthetic efficiency of the strains (PE) were calculated using Eq. (3) derived from Schipper et al. (2021):

$$PE = \frac{S_x \cdot \Delta H_C^0}{\frac{q_0 / E_{PAR}}{0.43}} \tag{3}$$

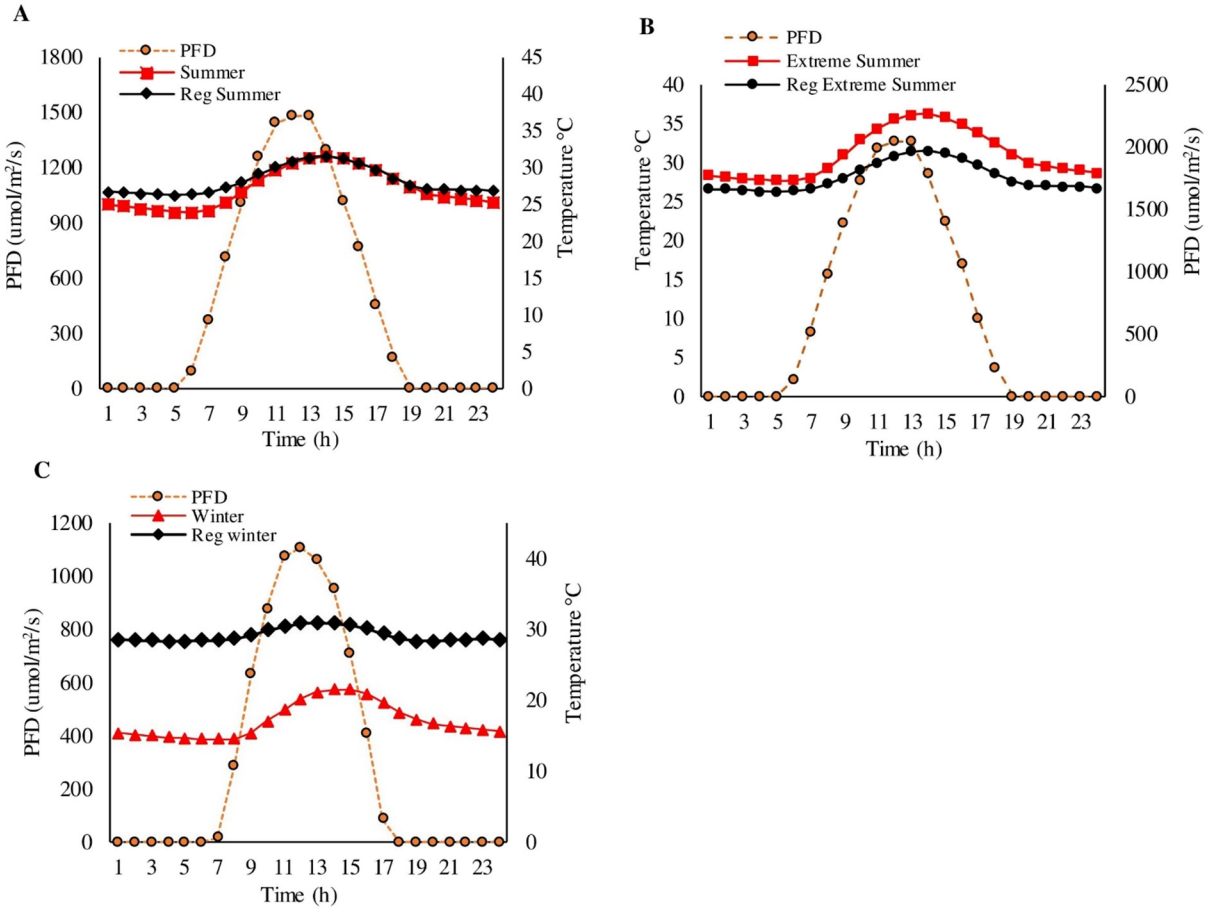


Fig. 1. Daily photon flux density (PFD) and culture temperature profile applied for (A) summer cultivation, with (reg. summer) and without thermal regulation (summer), (B) extreme summer cultivation with (reg. extreme summer) and without thermal regulation (extreme summer), and (C) winter cultivation with (reg winter) and without thermal regulation (winter). Only temperature values were altered between regulated vs non-regulated cultivations, PFDs remained the same.

where ΔH_C^0 is the enthalpy of biomass combustion (22.5 kJ/g), E_{PAR} is the average conversion factor of the PFD (q_0 in μmol/m^2/s) into an energetic flux calculated on the PAR (4.76 $\mu mol_h/m^2/s$ = 1 W/m^2) and 0.43 is the conversion factor from sunlight (full spectrum) to PAR light (43% of the total spectrum).

2.5. Biochemical composition

Total proteins, sugars and lipids were quantified using standard protocols that have been described in detail elsewhere (Rasheed et al., 2020). Briefly, 25 mg of freeze dried biomass was suspended in 5 mL of 0.1 M NaOH and hydrolyzed at 60 °C, overnight for protein extraction. The total protein content was determined using Folin ciocalteau reagent (Sigma-Aldrich). For the lipid quantification, repeated methanol chloroform extraction steps were done on biomass, to ensure complete removal of lipids. The organic phase was collected, dried and weighed to quantify the lipid content gravimetrically. For the carbohydrates, initially the biomass was pretreated using acetic acid and the mixture was placed in water bath at 85 °C, following which the pellet was completely decolorized using acetone. This step prevents interference by Chlorophyll (pigments) for the colorimetric assay. The pretreated colorless biomass was hydrolyzed using 4 M HCl, at 90 °C for 2 h, following which the supernatant was subjected to colorimetric assay using phenol sulfuric acid.

2.6. Fatty acid methyl esters (FAME)

FAMEs were extracted via a one-step transesterification method, where a known amount of freeze-dried biomass was treated, using sulfuric acid and methanol solution. The FAME fraction obtained was injected into Gas Chromatography (Shimadzu 2010 plus, Kyoto, Japan) and identified using SUPELCO standards obtained from Sigma-Aldrich (37 Component FAME Mix, St. Louis, MO, USA) (Bounnit et al., 2020).

2.7. Zebra fish toxicity tests

AB zebrafish embryos (wild type) were used to conduct all toxicity assays. Cultivation was done in 60× E3 media, prepared using NaCl (8.765 g), KCl (380 mg), $MgSO_4$ (1.19 g), and $CaCl_2$ (1.765 g) diluted in 0.5 L of MilliQ water. Adult fish were raised in 14:10 h light: dark cycles at a temperature of 28 °C (Al-Kandari et al., 2019). The fish were fed brine shrimps and/or dry food two times per day. Dry food consisted of Sparos Zebrafeed, purchased from Sparos (Olhão, Portugal).

Male and female adult fish were left overnight in a mating tank at a ratio of 3:2 separated by the tank divider. The following day, the dividers were removed, and the fish were left to mate for 5 h. Every 1–2 h, the eggs were collected, then viable fertilized eggs were selected and washed with E3 media and maintained in the incubator at 24 °C. All experiments conducted were consistent with the national and international guidelines for using zebrafish embryos in the experimental settings (Reed and Jennings, 2011). In addition, Qatar University's animal protocol guidelines was followed and the Policy on Zebrafish Research established by the Ministry of Public Health Qatar (Ministry of Public Health, 2017).

2.7.1. Acute toxicity and teratogenicity

Potential mortality and developmental toxicity of the microalgal biomass as feed was assessed with acute toxicity assays adapted from the

guidelines for testing chemical toxicity formulated by the Organization for Economic Co-operation and Development (OECD) (No. 203, 210 and 236) (OECD, 2013b, 2013a, 2019). A stock solution of 1.0 g/L of PM and NA were prepared by adding 0.01 g of each feed into 10 mL of fish media (E3 media). As a positive control, Dimethylaminobenzaldehyde (DEAB; Sigma-Aldrich (St. Louis, US)), a competitive inhibitor that causes significant mortality and teratogenicity was used (Cornet et al., 2017a; Abou-Saleh et al., 2019; Rasool et al., 2018; Younes et al., 2018).

Acute toxicity assay was performed on zebrafish embryos at 24 hours post-fertilization (hpf), the embryos were dechorionated in a small petri dish using 100 μL of 1.0 mg/mL pronase for approximately 10 min (Al-Jamal et al., 2020a; Al-Asmakh et al., 2020; Al-Kandari et al., 2019). After dechorionation, healthy embryos were placed in a 12-well plate and incubated in 3 mL of E3 containing i) 5 concentrations of PM Microalgae (20.0, 40.0, 80.0, 160.0, 320.0 mg/L), ii) 5 concentrations of the NA Microalgae (20.0, 40.0, 80.0, 160.0, 320.0 mg/L), iii) positive control (10 μM of DEAB), iv) E3 media (negative control 1), and v) 20 mg/L of Sparos ZebraFeed (negative control 2). The mortality rate was assessed for three consecutive days (48, 72, and 96 hpf) using a standard stereomicroscope microscope. The LC_{50} value was calculated by generating a sigmoidal curve to mortality rate at 95% confidence interval as described elsewhere (Nasrallah et al., 2018b; Al-Jamal et al., 2020b) using Graph Pad Prism 8 software (version 8.2.1, San Diego, USA). In this experiment, twenty-five embryos for each treatment condition were used.

For assessing the teratogenicity, zebrafish embryos (at 96 hpf) were imaged using a Zeiss Stereo Discovery V8 Microscope equipped with Hamamatsu Orca Flash high-speed camera and a workstation equipped with HCImage software, version 4.4.1.0 (Hamamatsu Photonics, Tsukuba, Japan) as described in previous studies (Al-Asmakh et al., 2020; Younes et al., 2018; Al-Jamal et al., 2020c). Variations in the body length, eye, heart, and yolk sac size were measured by Image J software version 1.52a (NIH, Washington DC) bundled with Java 1.8.0_172 (Al-Jamal et al., 2020a; Al-Asmakh et al., 2020).

2.8. Statistical analysis

For microalgae related experiments *t*-test and one way ANOVA (Tukey's post hoc Test) was applied for comparing results statistically (IBM SPSS, version 28.0). $P < 0.05$ was considered as a threshold for statistical significance. For survival rate in toxicity studies, Chi square test was used to assess the statistical significance between negative control and treated groups. For teratogenicity analysis, One-way ANOVA followed by Dunnett test was used to assess the statistical significance between treated groups in comparison to the negative control.

3. Results & discussion

3.1. Cultivation of microalgae in open ponds (resistance to contamination)

Prior to in depth investigation of the two microalgae PM and NA, their ability to grow in natural environment (NE), in open ponds was assessed for 16 days, as indicated in Fig. 2.

Both strains were able to grow well under outdoor conditions, after an initial lag-phase was observed. Final biomass concentrations after the batch cultivation were 1.19 ± 0.13 g/L and 0.86 ± 0.10 g/L for PM and NA, corresponding to areal productivities of 17.1 ± 2.0 g/m^2/d and 12.1 ± 1.6 g/m^2/d, respectively (Fig. 3).

Both local strains produced a higher biomass compared to *Nannochloropsis salina* (widely used strain for aqua culture) when cultivated in open ponds, exhibiting only 10.2 g/m^2/d (Uggetti et al., 2018). Additionally, no predator or contamination with phytoplankton was observed throughout the cultivation indicating the robustness and dominance of the two strains proving their suitability for outdoor cultivation. In a different study elsewhere, for cultivation of *Nannochloropsis* sp. in large scale, fresh water was used to prepare artificial sea water medium to avoid such contamination issues (Mohan et al., 2021). However, it should be noted that this is not economically feasible in places such as Qatar, where fresh water sources are limited. The difference in productivities for PM and NA was found to be insignificant ($p > 0.05$), hence it can be concluded that both strains are prospective candidates for large scale cultivation in ponds. They can withstand the temperature and light fluctuations encountered here and also resist any potential contaminations, that causes the cultures to crash outdoors.

3.2. Effect of seasonal variation on growth and composition of microalgae: response to environmental stimuli (light and temperature)

Following the outdoor experiment, the strains were cultured in lab-scale photobioreactors, to estimate year-round productivities under simulated temperature and light fluctuations from the described thermal model, representing seasonal variations in Qatar, under regulated and non-regulated conditions. Cultivation was performed under continuous mode, and all parameters (except light and temperature) were kept as non-limiting.

3.2.1. Biomass density and areal productivity

Microalgae regulate their growth and cellular mechanism as a response to different environmental stimuli by modulating their growth and biochemical profiles (Mc Gee et al., 2020). Several technoeconomic reports have suggested biomass productivity as the crucial criterion determining the commercial potential of any microalgae strain (Stephens et al., 2010; Yarnold et al., 2016). Therefore, a consistent and reliable estimation of biomass concentrations and productivities through continuous culture mode was implemented in this study, against batch cultures where adaptation and different growth phase will influence these parameters (Taleb et al., 2015). The areal productivities as a reliable parameter for outdoor culture, achieved for the different seasonal cultivation regimes are illustrated in Fig. 5. Samples for productivities were collected at the end of the light cycle, after the cultures have received maximum light exposure.

As the dilution rate was constant, there is a direct correlation between biomass concentration and productivities (see supplementary data for details on biomass concentration). For the non-regulated profiles, the highest biomass productivity found was 32.9 ± 2.5 g/m^2/d for PM under summer cultivation conditions corresponding to a biomass concentration of 0.44 ±

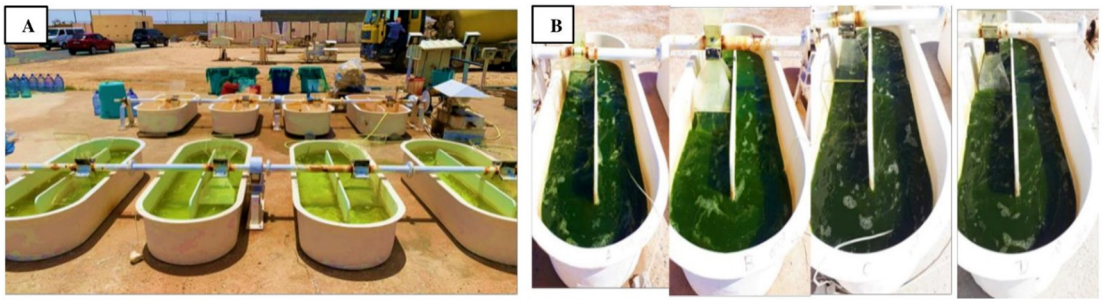


Fig. 2. Cultivation of the two strains in open raceway tanks. A represents the culture at $T = 0$, and B represents the culture at T = end. Both strains were cultivated in duplicates, $n = 2$.

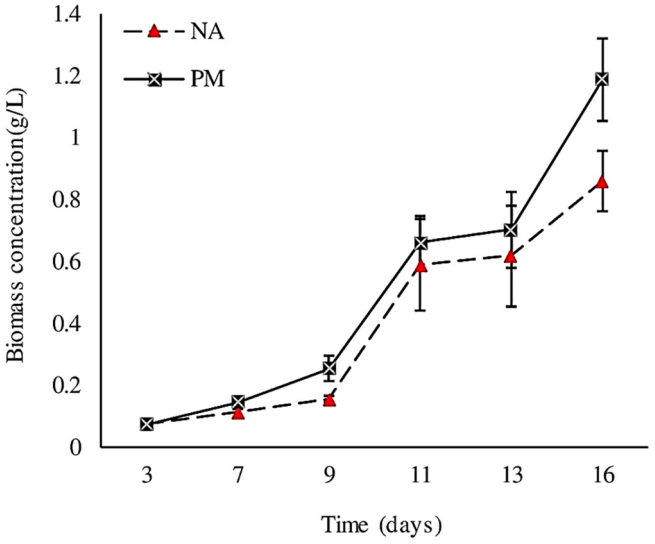


Fig. 3. Biomass concentration evolution for PM and NA for batch cultivation in open raceway tanks, under natural environment (NE). Data shown is the mean ± SD, $n = 2$.

0.03 g/L (supplemental material). Under extreme summer conditions however, as well as under winter conditions, the biomass productivities significantly ($p < 0.05$) declined to 15.06 ± 0.06 g/m^2/d and 10.76 ± 0.3 g/m^2/d, respectively. For NA, the strain exhibited its best growth during summer conditions as well, reaching a biomass productivity and concentration of 17.12 ± 0.8 g/m^2/d and 0.23 ± 0.02 g/L respectively. During winter, biomass productivity significantly declined to 9.05 ± 0.54 g/m^2/d, and during the extreme summer profile NA did not resist the high temperature and light intensities resulting in culture wash-out. Clearly, during summer, both strains showed their best growth. This trend concurs with the authors who demonstrated an increase in biomass in response to increase in light (Ho et al., 2012; Al Jabri et al., 2021). However, when the light and temperature were significantly high as under the extreme summer profile, the biomass productivities decreased drastically (i.e. P.M.) or the culture died (i.e. NA). The results are consistent with the general findings that light and temperature over a certain saturation threshold can limit cell growth. Although, under high light, some fast growing strains can produce high biomass by fulfilling their photon flux demand, while for others it can cause photoinhibition and reduce growth or induce cell death (Khoeyi et al., 2012).

3.2.2. Effect of thermal regulation

Thermal regulation of culture temperatures, both heating and cooling, can help support higher productivities, but can also come at a cost of up to 50% of total operational costs (Pruvost et al., 2019). Thermal regulation with limited power however has the potential to provide the benefits of increased productivities, without excessive expenditure. Such a means was proposed for raceway culture systems operated in Qatar desert by using a ground semi-buried heat-exchanger (Pruvost et al., 2019). Corresponding temperature profiles (i.e. regulated conditions) for summer, extreme summer, and winter conditions were applied during cultivation of both PM and NA (PFDs were unaffected), and resulting biomass productivities can be found in Fig. 4.

For the regulated winter condition, minimum culture temperatures were increased from 15.13 °C to 28.5 °C, leading to a biomass productivity increase of 76.6% (18.90 ± 0.32 g/m^2/d) and 32.9% (13.16 ± 1.93 g/m^2/d) for PM and NA, respectively. Under the regulated summer profiles, PM did not show a significant change in biomass productivity compared to the non-regulated scenario, whereas NA showed a significant increase of 25.7% (21.5 ± 0.5 g/m^2/d). Furthermore, while NA did not show sufficient growth to prevent wash-out under the non-regulated extreme summer conditions, under regulated conditions it reached a biomass productivity of 8.5 ± 0.7 g/m^2/d. These findings would suggest that the growth of NA is more affected by temperature than light under extreme conditions, exhibiting here the interest of thermal regulation through a semi-buried heat-exchanger. PM on the other hand showed no significant increase in productivity with regulation during extreme summer. This highlights the fact that for PM light intensities are a stronger inhibitor of growth than the temperatures during extreme summer.

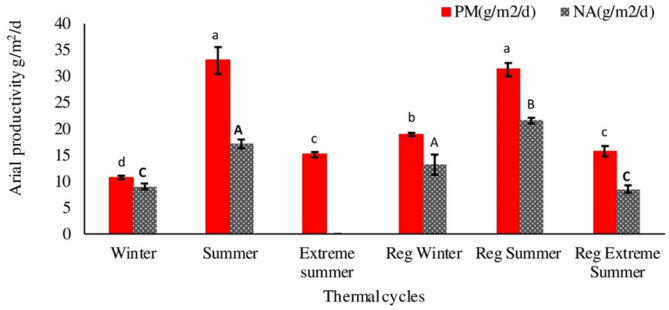


Fig. 4. Areal productivities (g/m^2/d) determined during steady state continuous cultivation (D = 0.017/h) for PM and NA under the different thermal regimes. Values are mean ± SD, $n = 6$, Different letters indicate significant differences ($p < 0.05$) between the individual means for NA and PM, respectively.

Results for both strains were in agreement with the prediction made by Pruvost et al., 2019, that limited thermal regulation (ground heat exchanger) can, particularly in winter, increase biomass productivities (Pruvost et al., 2019). However, it also demonstrates that the effect of thermal regulation on productivity is strongly strain dependent, as some strains are more sensitive to light intensities as compared to temperatures. Further, the effect of winter conditions on productivities is similar as found in other studies, where it was shown that sub-optimal temperatures and light intensities, reduced the capacity of algal cells to regenerate phosphate for photophosphorylation and as well as the activity of carboxylase enzymes, inhibiting normal growth of cells (Aburai et al., 2021). Additionally, during winter, the total light availability is lower as compared to summer due to lower light intensities and shorter days, resulting in lower biomass productivities (Holdmann et al., 2019).

The maximum productivities reached in this study, are close to practically achievable values lying between 20 and 35 g/m^2/d (Mohan et al., 2021). For example, in the case of *Nannochloropsis salina* (commonly used strain in aquaculture) for a semi continuous cultivation with daily harvests produced an average biomass of 34.4 g/m^2/d close to the productivity found in summer for PM (Mohan et al., 2021). On the other hand, NA exhibited a higher productivity than *Nannochloropsis oceanica*, as stated in another recent study, producing 8 g/m^2/d, nearly half the productivity found for our local strain (Saito et al., 2020).

Numerous studies have emphasized that lab scale experiments, such as in this study, can be used to assess industrial scale cultivation using simulations of comparable conditions of growth. These kind of screening approaches are not just cost effective and time saving but are reliable estimations (Saito et al., 2020). PM under extreme summer conditions here was found to produce the same amount of biomass both in open ponds and in lab-scale photobioreactors mimicking those conditions, 17.1 ± 2.0 g/m^2/d and 16.5 ± 0.4 g/m^2/d, respectively. This similarity suggests that our approach based on thermal modeling to predict outdoor conditions for given periods of the year is able to effectively simulate real operating conditions in open ponds, allowing then reliable estimations for further commercial exploitation. Such validations are of key significance for confirming their suitability for industrial processes.

Taken together, our results indicate that light and temperature fluctuations in open pond cultivation strongly affect the growth of microalgae during the year. While PM tolerated the extreme climatic variations of Qatar desert, NA showed lower tolerance. By implementing a ground heat exchanger, temperature fluctuations can be reduced, to promote improved growth of the microalgae strains in adverse conditions increasing the annual productivity of microalgae. While temperature regulation allows for

higher productivities and prevents growth inhibition, it will increase the energy demand and equipment requirements of the process (Pérez-López et al., 2017). As of such, operating heat exchangers will incur additional cost in the algae production process. To date there are limited studies into the techno-economic feasibility, however, Ryu et al. (2019) showed that heat-exchanger based temperature regulation in open raceway ponds resulted in a 44% increase in biomass productivity with an increase of 95% net profit compared to cultivation without temperature regulation. The key determinant for such analysis was found to be the improved biomass productivities. Additionally, such studies have also suggested benefits of using heat exchanger under harsher weather conditions to produce biomass all year round (Ryu et al., 2019). Therefore, a techno-economic evaluation for implementation of heat exchangers for year round cultivation of PM and NA is recommended.

3.2.3. Photosynthetic efficiency

Photosynthetic efficiencies (PE) are the one of the most important factors to be determined while producing any photosynthetic organism. Sufficient photosynthetic efficiencies are necessary to attain economically favorable biomass production at a large scale (Kumar et al., 2021). For techno economic evaluation of annual biomass production in Qatar, the yearly PE determination is then of interest.

Several factors govern the photosynthetic efficiencies of microalgae such as temperature, light, cell density, and environmental conditions and so on (Kula et al., 2017). Based on the seasonal light variation in Qatar PE was classified as shown in Fig. 5 A. PE1, PE2 and PE3 correspond to the photosynthetic efficiencies obtained for the different regimes (summer, extreme summer and winter) corresponding to the average daily irradiance

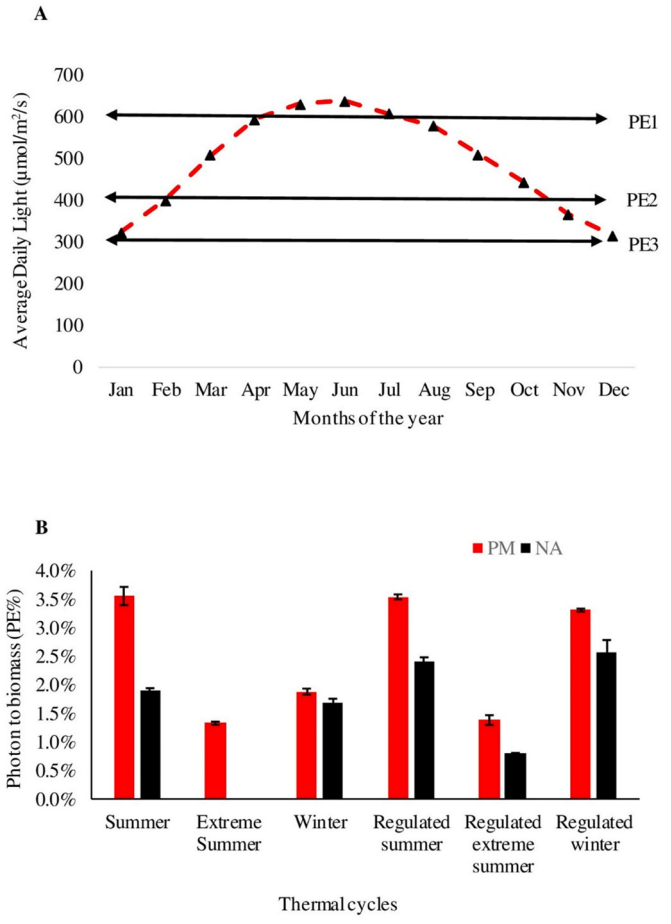


Fig. 5. (A) Yearly average light variation in Qatar and corresponding values used for PE calculation. B-Emergence of diverse PE (%) for two strains PM and NA over the main seasons in Qatar (with and without thermal regulation).

received by the cultures during the different seasons. The average daily light variation for the assessment of PE was based on a data published earlier (Schipper et al., 2021) while Fig. 5 B indicates the different values of PE achieved with and without regulation for the PM and NA.

The highest PE was observed during summer for both strains. PM exhibited a value of 3.54% while NA showed a significantly lower value of 1.89%. During winter, low light and temperature did not favor normal cell metabolism, negatively affecting the light to biomass conversion yield. Additionally, high light intensity with raised temperature also reduced the photosynthetic efficiency of the strains. In both scenarios, a higher variation in temperature negatively affected the cells. In this case, it can be hypothesized that temperature stress might have denatured proteins from the light harvesting complexes, greatly reducing the efficiency of the microalgae to utilize the available light. Previously, several strategies have been adopted to improve PE such as applying optimal growth conditions for temperature and medium, minimizing antenna sizes within the cells using genetic engineering, increasing turbulence, light dilution and so on (Melis, 2009; Kliphuis et al., 2010; Wijffels and Barbosa, 2010). Regarding culture conditions optimization, it was confirmed here that implementation of the ground heat exchanger can improve the biomass production, thereby increasing the photosynthetic efficiency for the strains. For NA the regulation significantly improved the PE by 16.8% during summer, whereas during winter an increase in PE by 43.8% and 27.1% was estimated for PM and NA respectively.

3.2.4. Nutritional potential of the biomass: metabolite content

A potential aquaculture feed ingredient is characterized by metabolic profile rich in proteins, lipids and carbohydrates as shown in Fig. 6.

Previously published studies mostly examined either the effect of light or temperature, and less well documented their interactive effects on metabolites (Guihéneuf and Stengel, 2017). Fig. 6 A, B, C describes the behavior of the strains at biochemical levels, during a typical day in summer and winter seasons of the year. Generally, the overall protein, lipids and carbohydrate contents for both strains were comparable with the range reported earlier for *Nannochloropsis* sp. with higher amount of lipids and proteins while carbohydrates were low ranging from 9 to 16% only (Lima et al., 2021). PM produced high proteins up to 54.5 ± 0.55% (*w*/w) during winter while in summer the protein content reduced to 40.2 ± 0.6% w/w. NA, on the other hand, produced 44 ± 2.25 (w/w) in winter compared to 38.7 ± 3.4 (w/w) in summer. It has been reported by several authors, at low solar irradiance as seen during winter, some microalgae respond strongly to the low light available, by densely packing the pigments to absorb light with the help of binding proteins (Lyon and Mock, 2014; Toseland et al., 2013). Furthermore, they also accumulate ribosomal proteins to counteract cold stress. They are also known to produce certain anti-freeze proteins categorized as lipo proteins and glyco proteins, with a global change in their encoded amino acids, however such deep investigation was not performed in this study (Aburai et al., 2021). Interestingly, the protein contents were lowered significantly ($P < 0.05$), during extreme summer in PM. Such a response has been reported previously, under high temperature exposure of cells, beyond the optimum, where the temperature unbalances the energy equilibrium within the cells and impairs photosynthesis. Excessive energy, which cannot be transformed, is removed, leading to the production of reactive oxygen species (ROS) (Barten et al., 2021). For lipids, both strains exhibited similar values with PM producing 29.5 ± 0.155% (*w*/w) lipids and NA exhibiting 28.65 ± 0.6% (w/w).

During summer, under high light regime, it is proved for several microalgae, that a simultaneous incr56ease in biomass and lipid accumulation is typical, as also evident for our strains (Morales-Sánchez et al., 2020b). Some studies suggest that abundant light promotes better lipid biosynthesis and accumulation whereas exposure to low light intensity (below the compensation point), causes reduced lipid biosynthesis (Zhu, 2015; Kumar et al., 2021). As observed for PM and NA, *C. subellipsoidea* C169 was also found to produce lipid droplets under heat stress such that lipids accumulated in the narrow range between 32 and 36 °C. Also, high temperatures direct the lipid biosynthesis into TAG accumulation (Morales-

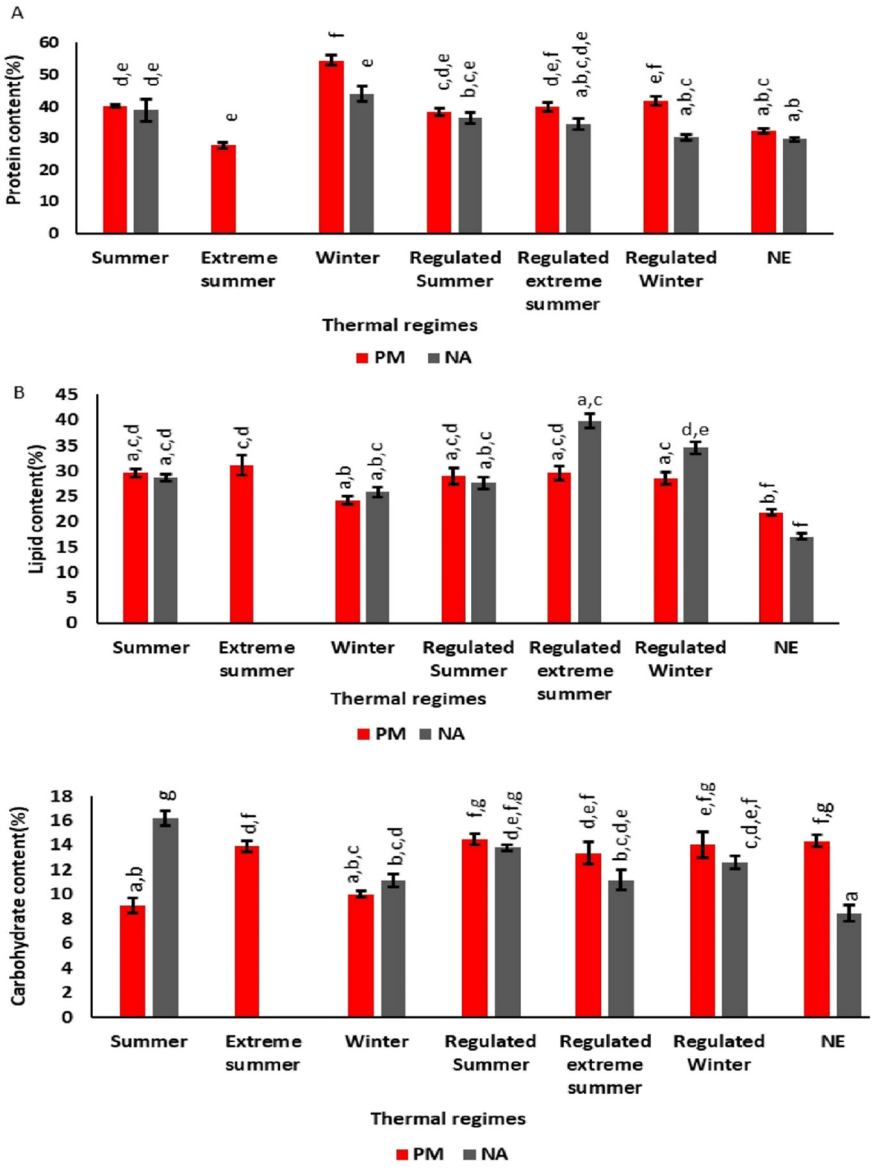


Fig. 6. A.B.C. Variation in biochemical composition of the two strains under different thermal regimes Summer, Extreme Summer, Winter (with and without the thermal regulation) and when cultivated in natural environment (NE). Protein, lipid and carbohydrate values are means ($n = 2$) ± SD. Different superscripts indicate significant differences.

Sánchez et al., 2020a). However, these values dropped during winter, and the strains accumulated more proteins as an act of acclimation to the extreme condition. Such kind of light and temperature adaptations have been reported for *Tetraselmis chui* in a different study where this genera exhibited high lipid and carbohydrate content which decrease with increasing protein contents during low light conditions (Lima et al., 2021). This correlation between proteins and lipids was also observed for PM during extreme summer. While the proteins were significantly low during extreme summer, lipids and carbohydrates were high up to 31.08 ± 1.98% (*w*/w) and 13.41 ± 0.458% (w/w) respectively. Increase in lipids and decrease in biomass also defines stress. For carbohydrates, NA accumulated higher sugars than PM during summer, which also accounts to the lower proteins and lipids found in NA under this regime. Overall, both strains had a lower carbohydrate content which is a strain dependent characteristic and can also be explained by the fact that carbohydrates are consumed during respiration and are also converted to lipids during night cycle, which was implemented during the night hours specified for our thermal cycles. (Raven and Beardall, 2003; Granum and Myklestad, 2002). Unlike, the biomass productivities, the thermal regulation did not project any remarkable effect on the metabolites. The strains continuously produced nutritionally valuable metabolites year-round and can be considered as a sustainable source of functional feed ingredient. Both strains indicated their characteristics as being semi thermophilic and psychrophilic microalgae. The production of metabolites per culture per day was also calculated from a commercialization perspective (see supplemental materials).

3.2.4.1. Assessment of fatty acid methyl esters. Apart from the primary metabolites, fatty acid composition is considered as a biomarker in the nutritional assessment of the microalgae strains (Guo et al., 2020). The Fatty acid profiling of the two strains was performed for the various seasonal conditions (see supplemental data for table).

Linolenic acid (ALA, C18:3, ω3) and Linoleic acid (LA, 18:2, ω6) were the highest fatty acids forming the PUFA fraction in the fatty acid profiles obtained for both strains. Similar abundance was reported for several microalgae, such as belonging to *Coccomyxa* species (Aburai et al., 2021). Numerous studies have documented that, ALA and LA are essential fatty acids, regarded as dietary precursors for the production of long chain w-6 fatty acids such as Arachidonic acid (ARA) and ω3 fatty acids such as

DHA and EPA respectively in fish. Many fish species such as Nile Tilapia, Murray Cod and Rainbow Trout have the ability to synthesize long chain PUFAs using these fatty acids as precursors (Chen et al., 2018).

An increase in ALA content from 20.21 ± 1.2 to 35.51 ± 3.4 mg/g and 45.84 ± 2.2 to 81.04 ± 7.71 mg/g was observed for PM and NA respectively, between summer and winter conditions. In a recent study, the highest ALA was recorded for *Chlorella* sp. reporting a concentration of 37.7 mg/g, while PM presented similar amount during winter, NA produced almost double this concentration producing up to 81.04 ± 7.71 mg/g at low temperatures. Similar increase was also seen for LA between the two seasons such that the strains produced higher total PUFA component in winter when compared to summer. Interestingly, NA also showed the presence of DHA in low amounts when cultivated in real operating conditions, in open ponds batch mode. Among, the saturated fatty acids, highest fraction was palmitic acid along with stearic acid. These fatty acids are not essential for feed purpose and hence not discussed further in detail.

Fig. 7, shows the variation in the relative abundance of saturated fatty acids (SFA), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). Overall, the PUFAs dominated the MUFA fractions. This is an important characteristic, as presence of high PUFA will improve the dietary value of the fish feed. The PUFA content was significantly high in both strains during winter compared to summer. These findings are in agreement with several studies, which highlight the association of low temperature and low light, with the production of PUFAs. Alternately, the lipid bilayer structure at the membrane of the cells is vulnerable to fluctuating temperatures. Hence, as a crucial adaptive strategy, microalgae rearrange the fatty acids in the membrane through an oxygen dependent enzyme, w3 desaturase, that sets unsaturated fatty acids in the bilayer to prevent it from losing its fluidity, flexibility and normal functionality, resulting in an increased PUFA content (Minhas et al., 2016). Similar mechanism have been well explained for Antarctic microalga *Chlamydomonas* sp. where desaturases are temperature regulated, at the transcriptional stage which modulate the fatty acid profile (Maneechote et al., 2021). Unsaturated fatty acids confer fluidity on acyl chains in cold environments. However, when temperature regulation was applied during winter, though it increased the biomass productivity, the PUFA content reduced insignificantly, from 58.25% to 47.85% and 51.79% to 46.17% for PM and NA respectively. During summer, both strains showed a decrease in PUFAs with a corresponding increase in SFA. Similar trend was found in literature, in which most species, including *Nannochloropsis*, *Rhodomonas* and *Isocrysis*, show an increase in fatty acid saturation with increasing temperature (Aussant et al., 2018; Renaud et al., 2002). However when temperature regulation was implemented during summer, NA showed a drastic improvement in PUFA content from 34.71 ± 1.5% to 50.15 ± 0.3%, with and without regulation respectively.

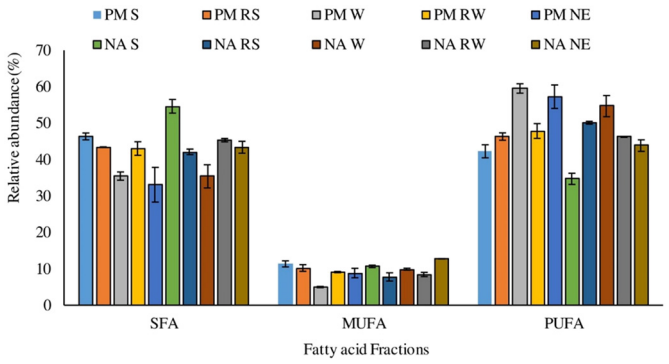


Fig. 7. Variation in the fatty acid content in terms of SFA (saturated fatty acid), MUFA (monounsaturated fatty acid) and PUFA (polyunsaturated fatty acid) for PM and NA under the different thermal regimes representing summer and winter seasons with and without regulation. S, RS, W, RW and NE indicate the different conditions implemented on the strains and they stand for summer, regulated summer, winter, regulated winter and natural environment (open pond cultivation) respectively.

In addition to the fatty acid content and their relative abundance, an appropriate ratio between omega 3 and omega 6 fatty acid is crucial for use as feed. It was found that PM and NA, under all culture conditions showed a low ratio within the expected range of 1:1 to 5:1 (data not shown), to be considered as beneficial for feed (Strobel et al., 2012). It is evident that both strains have the ability to synthesize essential fatty acids relevant to aqua culture and by implementing the thermal regulation, the fatty acid profiles can be optimized further.

3.3. Toxicity test on zebra fish embryos

Toxicity assays on zebra fish embryos is the first step towards application of these microalgal biomass as feed ingredient and determine their suitability in fish-rearing environments. The mortality rate of embryos, using PM and NA, was assessed at 24–96 hpf, which is the period where embryos are highly sensitive to drugs or chemicals (Yan et al., 2012a; Cornet et al., 2017c; Yan et al., 2012b; Wheeler et al., 2014; Hallare et al., 2004; Younes et al., 2018; Abou-Saleh et al., 2019; Nasrallah et al., 2018a). As egg chorion can act as a barrier, inhibiting chemicals from reaching the embryo, dechorionated zebrafish embryos were used (Zhao et al., 2015; Rieger, 2013). The percentage of cumulative survival was measured at 96 hpf and results are shown in Fig. 8 (Cornet et al., 2017b).

When applying PM and NA biomass concentrations of up to 320 mg/L, as well as in the control with conventional feed (ZebraFeed), no significant mortality was observed. On the contrary, the mortality rate following exposure to 10 μM of DEAB (the positive control) was 15%. PM and NA concentrations higher than 320 mg/L were not tested, as the E3 media got thick and opaque with the higher concentrations.

According to the mortality response curve of DEAB (Fig. 8 C), the calculated LC50 value for DEAB was 24.17 μM. Whereas, by means of the acute toxicity assays, exposure of zebrafish embryos to both strains of local microalgae (up to 320 mg/L) failed to elicit any signs of acute toxicity or mortality, suggesting a hypothetical LC50 much higher than the maximum dose. Alternately, as per the acute Toxicity Rating Scale provided by the USFWS, both strains were also described as “Practically non-toxic” and can be classified as “relatively harmless”, even if the tested concentrations were increased to 1000 mg/L.

3.4. Teratogenic effect by PM on zebrafish embryos

After evaluating the survival rate, the teratogenic effects induced by both strains on zebrafish embryos were assessed and recorded at 96 hpf. The exposition of embryos to 10 μM of DEAB elicited severe teratogenic deformities, such as heart sac edema, yolk sac edema, and shorter body length. On the other hand, PM and NA did not induce any significant morphological or physiological abnormalities on the zebrafish embryos at any dose and time employed (Fig. 9 A–D). Therefore, in concordance with the acute toxicity rules, these results provide another line of evidence that the micro algal strains used in this study are safe on the embryonic development of zebrafish embryos, thus can be used as potential ingredient in aqua culture feed. Future studies to determine the inclusion rate of the algae in the fish feed formulation can be performed for the strains through well-designed bioassay involving locally or internationally significant fish species.

4. Conclusion

The performance of two microalgae strains for annual large-scale commercial production in raceway ponds operated in harsh desert conditions was assessed, by using a climatic model to predict daily and seasonal variations in both temperature and light intensities, representing cultivation under Qatar's climate conditions. This kind of approach can be adapted to any geographical location having similarities in environmental and climatic conditions.

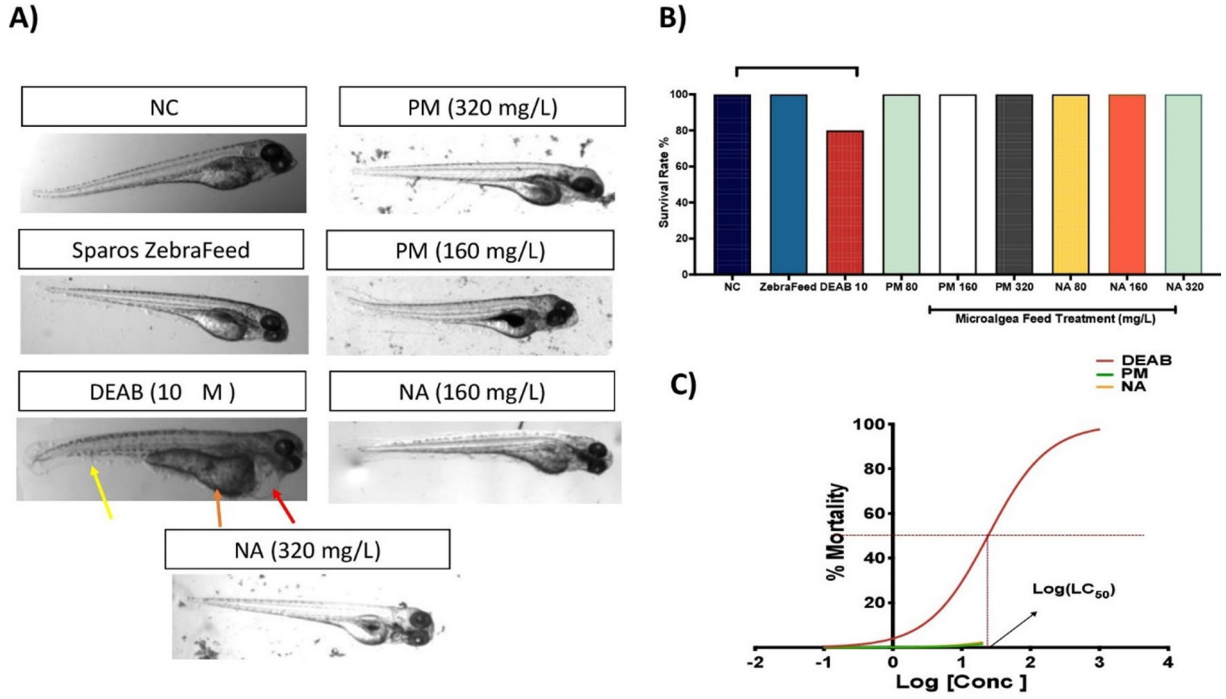


Fig. 8. (A) Representative photographs of 96-hpf embryos exposed to PTU medium (negative control), Sparos zebrafeed (negative control), DEAB nanoparticles (positive control), and all concentrations of Microalgae feed. The red arrow shows the heart edema, the orange arrow shows the yolk edema, and the yellow arrow shows the short body length. (B) The survival rate of the microalgae feed at 96 hpf. (C) Graph demonstrating the LC50 of Microalgae compared to the DEAB (PC). $n = 25$.

Both strains had high biomass productivities during the summer season, however reduced productivities were found for winter and extreme summer conditions. The application of thermal regulation proved beneficial, showing significantly improved biomass productivities under unfavorable conditions. This can promote continuous supply of biomass for feed production.

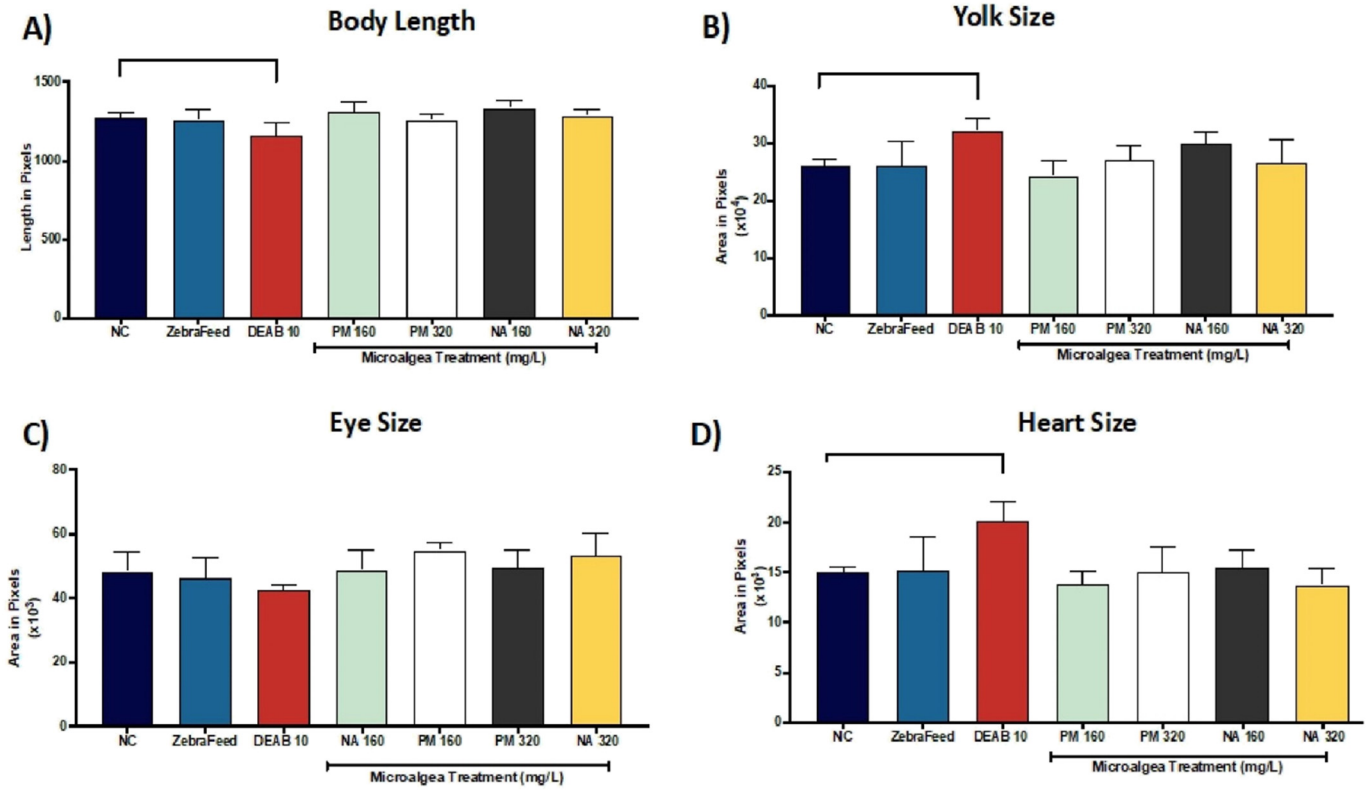


Fig. 9. A) Average body length, B) size of the yolk, C) size of the eye, and D) heart sac size following treatment, as captured using the HCImage software and analyzed with version 1.52a of the ImageJ software version. One-way analysis of variance (ANOVA) followed by the Dunnett test was used to compare the groups, $^{**}p < 0.01$ and $^{***}p < 0.001$, $n = 6$.

Furthermore, both strains were found to have superior nutritional potential with high proteins and lipids, including the presence of essential PUFAs throughout the annual seasonal fluctuations. Finally, with their zero toxicity against zebra fish embryos, they can be considered as safe at their highest concentrations for use as potential aqua feed ingredients in local and international markets.

CRediT authorship contribution statement

Rihab Rasheed: Investigation, Data curation, Writing – original draft, Writing – review & editing; **Mahmoud Thaher**: Microalgae cultivation set up (PBR and Pond), Investigation; **Nadin Younes**: Investigation, Data curation, writing and reviewing (Zebra fish assay); **Touria Bounnit**: Investigation-Lipid analysis (Quantitative and Qualitative); **Kira Schipper**: Manuscript - Review and editing; **Gheyath K. Nasrallah**: Methodology (zebra fish assay), Reviewing and Editing manuscript; **Hareb Al Jabri**: Funding acquisition, Manuscript - Review and editing; **Imma Gifuni**: Supervision, Data verification and validation, Manuscript - Review and editing; **Olivier Goncalves**: Supervision, Data verification and validation, Manuscript - Review and editing, **Jeremy Pruvost**: Conceptualization, Supervision, Data verification and validation, Manuscript-Review and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.155538.

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