Algal Research 61 (2022) 102564

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Towards a sustainable supply of omega-3 fatty acids: Screening microalgae for scalable production of eicosapentaenoic acid (EPA)

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ARTICLE INFO

Keywords:
Microalgae
Omega-3 fatty acids
Eicosapentaenoic acid
Photobioreactor
Scale-up

ABSTRACT

There is an increasing need for sustainable sources of omega-3 fatty acids; this challenge can be addressed through large-scale production of eicosapentaenoic acid (EPA) using microalgae. Identification of suitable strains is key in successful process scale-up. However, it is not certain whether conclusions from small-scale systems (i.e. flasks and well plates) can be translated to larger photobioreactors. To examine this issue the productivity and fatty acid composition of eleven different microalgal strains was quantified using both flask and photobioreactor cultures, generating a significant experimental dataset. Results from the flask screening offered relatively poor predictions of performance in photobioreactors, suggesting a need for improved screening tools. Of the species examined, *Phaeodactylum tricornutum* was found to be the most promising when grown in photobioreactors. To further guide scale-up the effect of environmental conditions (temperature and salinity) on EPA production was examined. It was found that the EPA content was ~5% of the dry biomass and this was approximately constant for the range of temperatures (13–27 °C) and salinities (35–50 g L^{-1}) examined. Finally, detailed nutritional information about the biomass is presented, which can serve as a starting point for its formulation into food products.

1. Introduction

Eicosapentaenoic and docosahexaenoic acids (EPA and DHA) are essential nutrients for humans due to their vital roles in neurological development and prevention of chronic diseases [1–4]. The main dietary source of EPA and DHA is fish [5–7]. However, it has been estimated that the current supply of EPA and DHA from both capture fisheries and aquaculture could meet only 30% of the demand for human consumption (1.3–1.4 Mt yr^{-1}) [7,8]. The growing world population along with the stagnation in global fish stocks could further exacerbate the EPA/DHA shortfall [7]. Sustainable strategies are clearly needed to bridge the EPA/DHA supply-demand gap.

One way of addressing this challenge is the heterotrophic cultivation of microorganisms which produce EPA and DHA. For example, *Schizochytrium* sp. and *Crypthecodinium cohnii* [9] as well as recombinant strains of the yeast *Yarrowia lipolytica* [10] have been used for the commercial production of EPA and DHA. Heterotrophic processes have the advantage of relatively high productivities, particularly when compared to photoautotrophic processes. However, they rely on plant-based feedstocks, which may have drawbacks from a sustainability perspective. In contrast, photoautotrophic production does not require arable land or fresh water, which may be advantageous from a sustainability perspective [11]. Development of scalable, cost-effective production processes is the factor limiting the photoautotrophic production of EPA using microalgae. A key factor is the identification of suitable species for large-scale production. Suitable species would have a high EPA content, the ability to be readily cultivated at scale and have a composition such that they can be readily included in foods.

Numerous studies have characterized the fatty acid profiles of microalgae, finding that certain species of Haptophyta, Bacillariophyta, Ochrophyta and Rhodophyta contain EPA up to 30–50% of the total fatty acids [12–16]. However, the majority of previous work has been done using flask cultures and it is uncertain whether these results can be extrapolated to large systems. Conditions in large-scale photobioreactors are more complex than those found in small-scale screening systems, for example, the cells might be subject to fluctuations in light conditions and hydrodynamic stress of pumping [17]. Additionally, outdoor growth imposes additional stresses on the cells, for example,

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https://doi.org/10.1016/j.algal.2021.102564
Received 16 July 2021; Received in revised form 29 October 2021; Accepted 10 November 2021
Available online 24 November 2021

Table 1
Summary of growth conditions used in the 5 L flat panel photobioreactors as well as the outcome of the cultures.

Phylum	Class	Species/strain	Growth conditions in photobioreactors				Outcome
			Medium	Temperature & salinity	Light	Air flow	
Orchrophyta	Bacillariophyceae (pennate diatoms)	*Nitzschia paleacea* CS-430	f/2	20 °C, 35 g L^{-1}	100 μmol m^{-2} s^{-1}	5 L min^{-1} with 1% (v/v) CO_2	No growth
		Phaeodactylum tricornutum CS-29	f/2	20 °C, 35 g L^{-1}	150 μmol m^{-2} s^{-1}	5 L min^{-1} with 1% (v/v) CO_2	Consistent growth
	Coscinodiscophyceae (centric diatoms)	*Chaetoceros* sp. CS-256	f/2	30 °C, 35 g L^{-1}	150 μmol m^{-2} s^{-1}	5 L min^{-1} with 1% (v/v) CO_2	Consistent growth
		Skeletonema sp. CS-1112	f/2	20 °C, 35 g L^{-1}	150 μmol m^{-2} s^{-1}	5 L min^{-1} with 1% (v/v) CO_2	Consistent growth
	Eustigmatophyceae	*Microchloropsis salina* CS-190	f/2	Not tested in PBRs			
		Nannochloropsis oculata CS-179	f/2	23 °C, 35 g L^{-1}	100 μmol m^{-2} s^{-1}	5 L min^{-1} with 1% (v/v) CO_2	Consistent growth
		Nannochloropsis oculata CS-192	f/2	23 °C, 35 g L^{-1}	100 μmol m^{-2} s^{-1}	5 L min^{-1} with 1% (v/v) CO_2	Inconsistent growth
Haptophyta	Pavlovophyceae	*Pavlova lutheri* CS-182	f/2	20 °C, 29 g L^{-1}	100 μmol m^{-2} s^{-1}	5 L min^{-1} with 1% (v/v) CO_2	Inconsistent growth
		Rebecca salina CS-49	f/2	Not tested in PBRs			
Rhodophyta (red algae)	Porphyridiophyceae	*Porphyridium purpurem* CS-25	Jones'	23 °C, 35 g L^{-1}	100 μmol m^{-2} s^{-1}	5 L min^{-1} with 1% (v/v) CO_2	Grew in biofilms
	Stylonematophyceae	*Rhodosorus* sp. CS-249	f/2	27 °C, 35 g L^{-1}	100 μmol m^{-2} s^{-1} (Days 0–6) 150 μmol m^{-2} s^{-1} (Days 7–14)	10L min^{-1} with 1% (v/v) CO_2	Consistent growth

they will be exposed to diurnal and seasonal temperature variations which can affect both the growth rate and fatty acid composition [18–24]. Open culture systems (i.e. ponds and raceways) while having the advantage of lower capital costs are also particularly susceptible to contamination. Strains that can tolerate hypersaline conditions may be advantageous in this aspect as increasing the salinity of the growth medium has been shown to be a viable strategy for reducing microbial contamination [25]. Hence, there is a need for additional work to characterize the performance of strains to have a better understanding of how they would perform under relevant, industrial conditions.

Recent years have seen an emerging trend in algal functional foods and supplements. *Chlorella* sp. and *Arthrospira* sp. were among the first microalgae/cyanobacteria that were commercialized as dietary supplements; however, they lack essential ω-3 fatty acids [9]. As previously noted, inclusion of EPA containing algal biomass in foods is a way to sustainably provide these key nutrients. Understanding the whole biomass composition (and not just the fatty acids in isolation) is a necessary step prior to inclusion in foods, particularly since the biomass may provide other valuable nutrients (e.g. protein and vitamins). Zanella and Vianello [26] reviewed the composition of *Nannochloropsis* species, finding that in addition to EPA the biomass contained relatively high levels of cobalamin (vitamin B_{12}) and carotenoids. Knowledge of the detailed nutritional composition of other species will be essential in guiding their inclusion into food (and feed) products.

Hence, the aims of this work are threefold: i) to quantify the fatty acid composition and biomass productivity at both flask and photobioreactor scale for a range of species and hence determine whether results from small-scale systems can be applied at larger scales; (ii) investigate the effects of temperature and salinity changes on the most promising strain with the aim of identifying the range of conditions which could be feasibly used for its large-scale production; and (iii) carry out a detailed nutritional analysis of its biomass.

2. Methods

2.1. Algal cultures and growth media

Eleven species of marine microalgae were acquired from the Australian National Algae Culture Collection (ANACC). These strains represent six classes from three phyla; information about the strains used is given in Table 1.

Stock cultures were maintained in f/2 medium (composition detailed below) in Erlenmeyer flasks with working volumes of 15–25 mL, placed on a cool-white-light LED pad with a 12:12 h light:dark photoperiod and a light intensity of ~60 μmol m^{-2} s^{-1}. Light intensity was measured using a universal light meter (ULM-500, Walz GmbH, Effeltrich, Germany). Temperature was in the range 18–26 °C. The stock cultures were passaged to fresh medium every three to four weeks.

All flask and photobioreactor cultures in the study were grown in f/2 medium, except for the photobioreactor culture of *Porphyridium purpurem*. *P. purpureum* was grown in modified Jones' medium, which was optimized for the growth of *Porphyridium* sp. [27]. The media were prepared at seawater salinity by adding 35g $L^{-1}$of a marine salt mix (Quantum® Mixed Macro Probiotic Salt™, Quantum Aqua, Australia) in deionized water. To this macronutrient, trace metal and vitamin stock solutions were added. The composition of f/2 medium used was $NaNO_3$ (880 μM), NaH_2PO_4 (36 μM), $NaSiO_3$•$5H_2O$ (140 μM), $FeCl_3$•$6H_2O$ (12 μM), $CuSO_4$ (41nM), $ZnSO_4$ (76 nM), Na_2MoO_4 (37 nM), $CoCl_2$ (37 nM), $MnSO_4$ (940 nM), disodium EDTA (12 μM), thiamine hydrochloride (300 nM), biotin (2 nM) and cyanocobalamin (0.4 nm). The composition of modified Jones' medium was $NaNO_3$ (10mM), K_2HPO_4 (0.5 mM), $MgSO_4$•$6H_2O$ (1 mM), $NaHCO_3$ (460 μM), H_3BO_3 (10μM), $FeCl_3$•$6H_2O$ (14 μM), $MnCl_2$•$4H_2O$ (364 nM), $ZnSO_4$•$7H_2O$ (153 nM), $CoCl_2$•$6H_2O$ (84.1 nM), $CuSO_4$•$5H_2O$ (80.1 nM), Na_2MoO_4•$2H_2O$ (49.6 nM), disodium EDTA (27.1 μM), thiamine hydrochloride (300 nM), biotin (2 nM) and cyanocobalamin (0.4 nM). All chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA), with the exception of biotin and cyanocobalamin which were purchased from Sapphire Bioscience (Sydney, NSW, Australia).

2.2. Flask screening

Strain screening was first carried out in 500 mL Erlenmeyer flasks with a working volume of 300 mL. f/2 medium was used for all cultures. The medium was autoclaved for 25 min and allowed to cool to room temperature before being inoculated with 3 mL of stock culture. The cultures were maintained under the same conditions as those for the stock cultures. Measurements of optical density (OD) and nitrate concentration were performed daily using a Varian Cary 50 spectrophotometer (Varian, Mulgrave, VIC, Australia). All cultures were harvested

for determination of the final biomass concentration and fatty acid analysis towards the end of the exponential phase – either on the day where the nitrate was exhausted or the OD did not increase for two consecutive days, whichever occurred first.

2.3. Photobioreactor screening

Strain screening was also carried out in 5-L flat-panel photobioreactors. The photobioreactors were constructed from clear acrylic sheets and had the dimensions of 600 mm (high) × 200 mm (wide) × 50 mm (deep). Temperature regulation was achieved by circulating water through a stainless-steel coil submerged in the culture medium. Agitation was provided by introducing air enriched with 1% (v/v) CO_2 (Food Grade, BOC, Australia) from the bottom of the photobioreactors through a 0.25 mm diameter stainless-steel tube with evenly spaced 1 mm holes. Light was provided unidirectionally by cool-white-light LED bars (9 W, 6000 K colour temperature, Jaycar, Australia) at a 12:12 h (light:dark) photoperiod. A schematic diagram of the photobioreactors is given in our previously published work [28].

Inocula used for the photobioreactor cultures were grown for 10–14 days under the same conditions as those for maintaining the stock cultures. The photobioreactor cultures were inoculated with a volume of 100–300 mL to give an initial OD of 0.03–0.05 at a wavelength of 550 nm. The batch cultures were cultivated for a duration of 14 days, except for *Chaetoceros* sp. A shorter length of time was used for *Chaetoceros* sp. as the cultures reached stationary phase after 6–7 days of growth. Samples were collected for optical density and nitrate concentration measurements every 1–2 days, and for dry cell weight measurement and fatty acid analysis every second day starting on Day 6 or 8. The sampling point was thoroughly flushed before sampling. Losses in culture volume due to evaporation (typically in the order of 50–120 mL per day) and sampling were replaced with deionized water such that the total liquid volume was maintained at approximately 5 L. Air flow was provided at 10 L min^{-1} for *Rhodosorus* sp. and 5 L min^{-1} for the other strains. This was done as the *Rhodosorus* sp. tended to form clumps which would fall out of suspension at the lower air flow rate. Attempts were initially made to grow all the strains at constant conditions of 20 °C and 150 μmol m^{-2} s^{-1}. However, many of the strains did not grow under these conditions. Hence, both the temperature and light intensity were varied in order to achieve growth, with the conditions used being given in Table 1.

2.4. 50 L bubble column cultivation for nutritional analysis

Algal biomass for the nutritional analysis was grown in a pilot-scale (50 L) bubble column photobioreactor, with design of the reactor being detailed in our previous work [29]. To boost the biomass concentration, concentrated growth medium was prepared with the nitrate and phosphate being added at 15 × f/2 concentrations and the other components being added at 5 × f/2 concentrations. Air was introduced at a flowrate of 20 L min^{-1} (0.4 vvm), enriched with 1% (v/v) CO_2. Temperature was maintained at 22 ± 1 °C. To avoid potential photoinhibition during the lag phase, light was supplied at an intensity of 230 μmol m^{-2} s^{-1} on the day of inoculation, and the light intensity was increased to 600 and 1100 μmol m^{-2} s^{-1} after ~24 h and ~96 h, respectively. The lighting was operated at a photoperiod of 16:8 h (light:dark). The sample was harvested on Day 15 at a biomass concentration of approximately 1.5 g L^{-1}. Approximately 90% of the nitrate in the growth medium had been consumed by the algae. The sample was dewatered using a WVO Raw Power centrifuge (WVO Designs, North Charleston, SC USA). The resultant biomass paste (containing ~77% water) was packed on ice and shipped to ALS Global (Melbourne, VIC, Australia) for analysis. Full copies of the analysis reports are provided in the Supplementary Material.

2.5. Analytical methods

Optical density was measured at a wavelength of 550 nm using a Cary 50 spectrophotometer with a 1 cm path length. Samples were diluted where necessary such that the absorbance was <0.8.

To measure the dry cell weight, a known volume of sample (typically ~50 mL) was collected from the culture and filtered through pre-weighed 0.55 μm filter paper (Advantec GA-55, Toyo Roshi Kaisha Ltd., Tokyo, Japan). The filter paper was then washed with three volumes of 0.5 M ammonium bicarbonate solution to remove the salts in the medium. Subsequently, the filter paper was dried overnight in an oven at 105 °C. Biomass productivity was determined as:

$$Biomass\ productivity\ \left(mg\ L^{-1}\ day^{-1}\right) = \frac{DCW}{t} \tag{1}$$

where *DCW* is the dry cell weight (mg L^{-1}); *t* is the time (day) since Day 0.

Nitrate concentration in the medium was measured using a Cary 50 spectrophotometer at wavelengths of 220nm and 275 nm [30]. Prior to analysis, the aliquot was filtered through a 0.2 μm PTEE syringe filter (Advantec, Toyo Roshi Kaisha Ltd., Tokyo, Japan) and was diluted such that the absorbance was <0.8. The nitrate concentration (mg L^{-1}) was calculated as following:

$$\begin{cases} \left[NO_3^-\right] = 0 \quad OD_{220nm} < 10{\cdot}OD_{275nm} \\ \left[NO_3^-\right] = \dfrac{OD_{220nm} - 2 \times OD_{275nm}}{0.06335} D_{220nm} \geq 10{\cdot}OD_{275nm} \end{cases} \tag{2}$$

Fatty acids were quantified using gas chromatography. Samples collected from the cultures were centrifuged at 6000 RCF for 5 min. The supernatant was discarded, while the pellet was resuspended in deionized water. The centrifugation and resuspension were repeated twice to remove the salts. The remaining pellet was freeze dried. Total lipid extraction was used a Bligh and Dyer method as per Breuer et al. [31,32]. A known amount of freeze-dried biomass powder (~10 mg) was added to a bead-beating tube preloaded with 0.5 mm high-impact zirconium beads (Sigma Aldrich). Milli-Q water, chloroform and methanol were added at the ratio of 8, 10 and 20 μL per mg of dry biomass, respectively, with glyceryl triheptadecanoate (Sigma Aldrich) being added as the internal standard at the concentration of ~1.2 mg mL^{-1} chloroform. The sample was bead-beaten at 4000 rpm using a BeadBug (PathTech, Preston, VIC, Australia) for 1 min and then transferred to a glass vial. Milli-Q water (20 μL per mg dry biomass) and chloroform (20 μL per mg dry biomass) were then added. The sample was vortexed and allowed to stand for 10–20 min to allow phase separation. The bottom (chloroform) layer containing the lipids was aspirated. The top layer was washed with chloroform (40 μL per mg of dry biomass), and the chloroform extracts were pooled. The pooled extracts were allowed to stand overnight for chloroform to evaporate. The bead-beating procedure was omitted for *Phaeodactylum tricornutum* CS-29 and *Nannochloropsis oculata* CS-179 as experiments showed that the omission of this step did not yield significantly different results for these two strains.

Transesterification of the extracted lipids was done by adding 3 mL methanol containing 5% (v/v) concentrated (99%) sulfuric acid to the dried sample. The sample was then heated in a water bath at 70 °C for 3 h, during which, the sample was vortexed every 30 min. Subsequently, the sample was cooled to room temperature, followed by the addition of 3 mL Milli-Q water and 3 mL *n*-hexane (chromatography grade, Sigma Aldrich). The sample was then allowed to stand to facilitate phase separation. One millilitre of supernatant from the top (hexane) layer was removed and transferred to a GC vial for analysis.

The fatty acid methyl esters (FAMEs) were analysed using a gas chromatograph (Shimadzu GC2010 Plus, Kyoto Japan) equipped with a 30 m long FAMEWAX column (fused silica capillary column, polar phase Crossbond polyethylene glycol, internal diameter 0.32 mm, film thickness 0.25 μm, Restek) and a flame ionization detector. The sample was

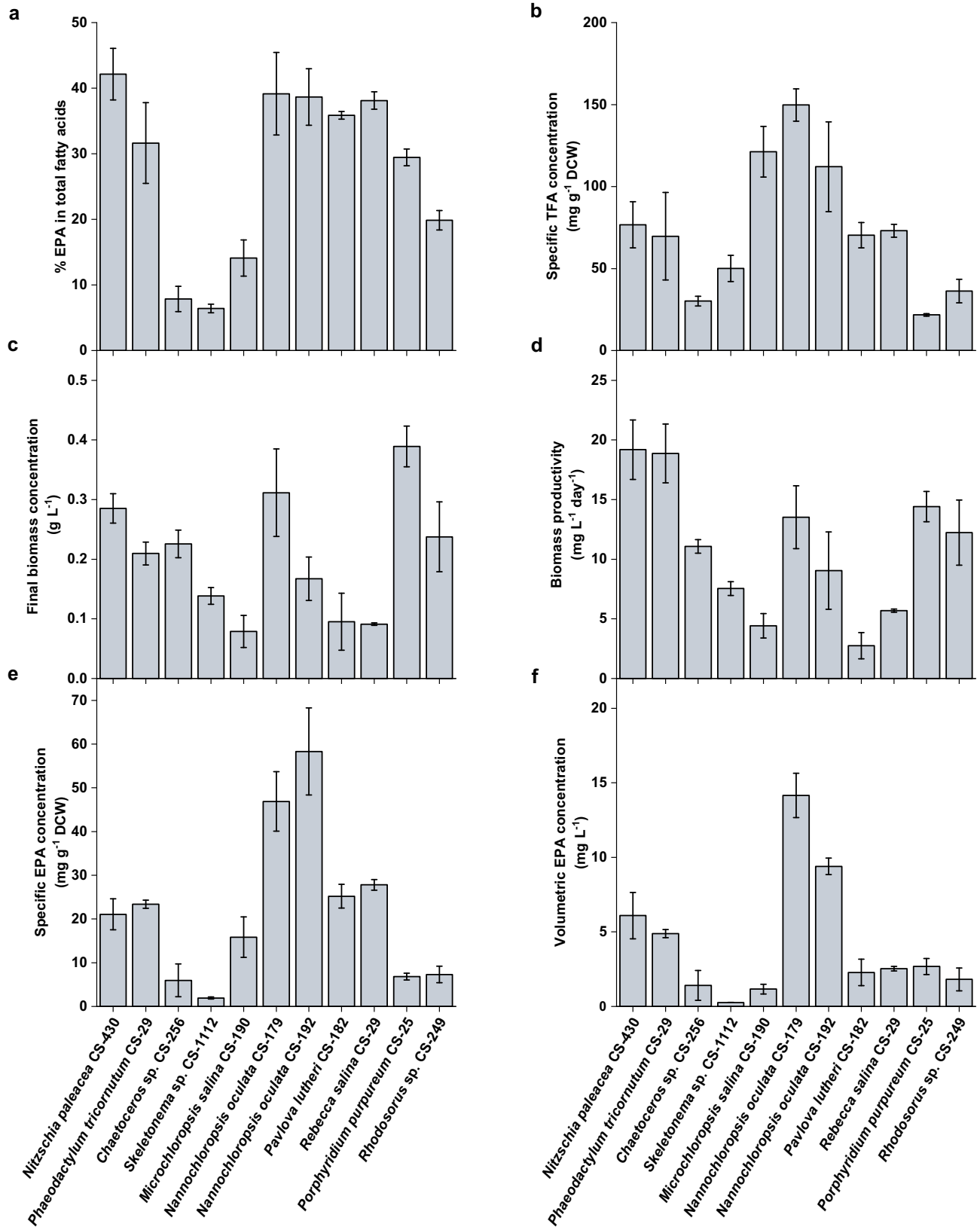

Fig. 1. Screening microalgae in flask cultures. (a), Percentage of EPA as a fraction of total fatty acids. (b), Specific total fatty acid (TFA) concentration per microalgal dry cell weight (DCW). (c), Final biomass concentration. (d), Biomass productivity. (e), Specific EPA concentration per microalgal DCW. (f), volumetric EPA concentration per L of culture broth. Bar charts represent the mean of the three biological replicates with error bars denoting one standard deviation about the mean.

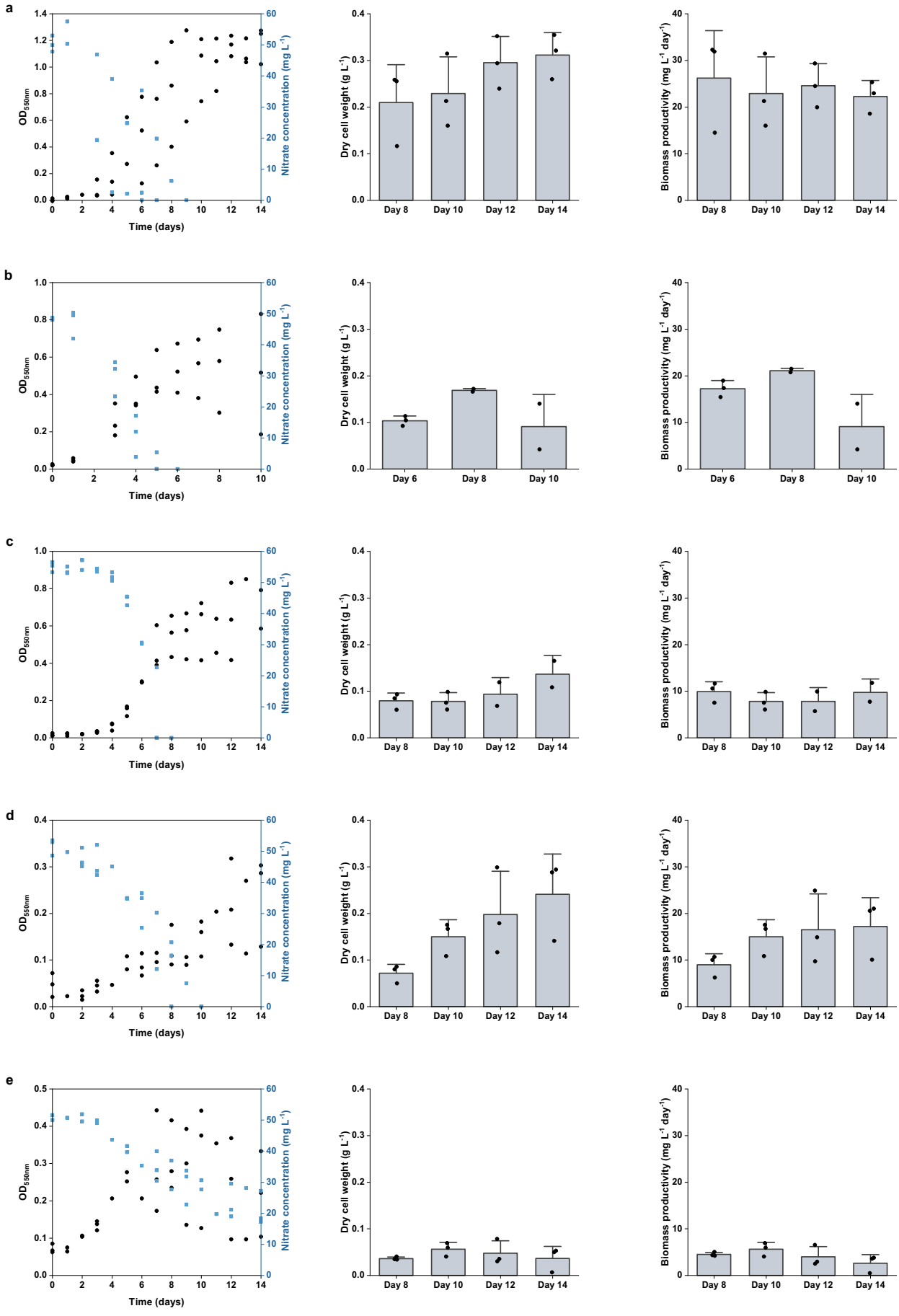

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Fig. 2. Growth in photobioreactors. (a–e), Changes in optical density at 550 nm, nitrate concentration, dry cell weight and biomass productivity as a function of culture time in 5-L flat-panel photobioreactors for microalgal strains *Phaeodactylum tricornutum* CS-29 (a), *Chaetoceros* sp. CS-256 (b), *Skeletonema* sp. CS-1112 (c), *Rhodosorus* sp. CS-249 (d), and *Nannochloropsis oculata* CS-179 (e). Bar charts represent the mean of the three biological replicates with error bars denoting one standard deviation about the mean; scatter plots represent all biological replicates (a–e). Dry cell weight and biomass productivity of *Cheatoceros* sp. CS-256 on Day 10 and *Skeletonmea* sp. CS-1112 on Days 12 and 14 are calculated with two replicates because the third replicate did not contain sufficient biomass for measurement (b, c).

injected into the column at an injector temperature of 250 °C and an injection volume of 5 μL with helium (BOC, Australia) as the carrier gas at a velocity of 0.25 m s^{-1} and a split ratio of 25:1. The column temperature was programmed to increase from 130 °C to 230 °C at a 5 °C min^{-1} increment during the first 20 min, then held at 230 °C for another 20 min. To identify the fatty acids, relative retention times of the FAMEs were compared with those of a reference FAME standard (Supelco 37 component FAME mix, purchased from Sigma Aldrich). The specific concentrations of the individual fatty acids (mg g^{-1} DCW) were calculated as:

$$FA\ concentration = \frac{IS \cdot A_{FA}}{A_{IS} \cdot M_{biomass} \cdot RF_{FA}} \quad (3)$$

where *IS* is the amount of internal standard added into the sample (mg per sample); $M_{biomass}$ is the amount of dry biomass in the sample (g per sample); A_{FA} is the area of the GC peak of the FAME for the fatty acid;A_{IS} is the area of the GC peak of the internal standard; RF_{FA} is the relative response factor of the FAME.

The specific concentration of total fatty acids (TFA) (mg g^{-1} DCW) was calculated as the sum of the concentrations of all the individual fatty acids.

EPA productivity (mg L^{-1} day^{-1}) was determined as:

$$EPA\ productivity = \frac{specific\ EPA\ concentration \times DCW}{t} \quad (4)$$

2.6. Statistical analysis

All experiments were conducted in three independent replicates, except for the nutritional analysis, which was carried out in one repeat. The values of optical density were reported for each replicate, whereas the reported values of biomass productivity, specific fatty acid concentration and EPA productivity were presented in bar graphs showing the mean with error bars representing one standard deviation. Effects of salinity were analysed using Student's *t*-test (two-tail, $\alpha < 0.05$), performed with Microsoft Excel 2008. Effects of temperature were analysed using one-way analysis of variance with Tukey test used to compare the means ($p < 0.05$), performed with MATLAB R2019b.

3. Results

3.1. EPA content of microalgae

EPA was found to be present in all eleven strains examined in the flask-screening, with there being considerable (6 to 39%) variations in the percentage of EPA as a fraction of total fatty acids (Fig. 1a and Supplementary Table 1). The fatty acid profiles obtained here for the eleven strains are consistent with the findings reported by other authors [14,33]. There was also considerable variation in the specific EPA concentration (2 to 58 mg g^{-1} dry cell weight) (Fig. 1e and Supplementary Table 1). Some species (e.g. the red algae *Porphyridium purpureum* and *Rhodosorus* sp.) had a relatively high fraction of EPA but low specific EPA concentrations due to their low content of fatty acids (Fig. 1b). Use of an alternative growth strategy may lead to increased specific TFA concentrations. However, this approach was not examined as EPA is unlikely to be used as a storage compound [34].

The highest volumetric EPA concentrations were observed in *Nannochloropsis oculata* CS-179 and CS-192, being in the range of 9 to 14 mg L^{-1}, followed by those (5 to 6 mg L^{-1}) of *Nitzschia paleacea* and *Phaeodactylum tricornutum* (Fig. 1e). Although *Pavlova lutheri* and *Rebecca salina* had high specific EPA concentrations, their volumetric EPA concentrations were low (2 to 3 mg L^{-1}), due to their low biomass accumulation in the flask cultures (Fig. 1c, d). Interestingly, *Microchloropsis salina*, despite being in the same class as *Nannochloropsis* sp., showed a volumetric EPA concentration (~1 mg L^{-1}) among the lowest (Fig. 1f).

3.2. Photobioreactor screening

To examine the suitability of the microalgae for large-scale cultivation, nine of the eleven strains were subjected to a screening process in flat-panel photobioreactors, a commonly used closed system for phototrophic cultivation of microalgae. *M. salina* and *R. salina* were not screened in the photobioreactors because their taxonomically related strains, *N. oculata* and *P. lutheri*, respectively, had showed similar or better performance in the flask cultures.

As summarized in Table 1, of the nine species examined five grew reproducibly in the photobioreactors, two species grew with a large degree of variation between replicates (Supplementary Fig. 1), *P. purpureum* formed biofilms and no growth was observed for *N. paleacea.*

After being introduced to the photobioreactors, the cultures typically went through a lag phase lasting for 1–3 days. For *P. tricornutum*, *Chaetoceros* sp., *Skeletonema* sp. and *Rhodosorus* sp., the lag phase was followed by an exponential and/or linear phase that typically ended 1–4 days after the depletion of nitrate in the culture broth (Fig. 2a–d). These four strains all showed varying ability to grow on internal nitrogen pools, a phenomenon that has been observed in many algae [35,36]. The specific EPA concentrations of *Chaetoceros* sp., *Skeletonema* sp. and *Rhodosorus* sp. were <10 mg g^{-1} of DCW, whereas the specific EPA concentration of *P. tricornutum* remained at 40 to 50 mg g^{-1}. For the species examined the specific EPA concentration was not observed to increase with time, implying that EPA is not used as a storage compound, a finding consistent with the literature [34]. *P. tricornutum* was the only species where the TFA content was found to increase with time (Fig. 3 and Table 2). The accumulation of storage lipids was believed to be induced by nitrogen limitation in *P. tricornutum* [37]. In the photobioreactor cultures, the specific EPA concentration of *P. tricornutum* (in the range of 40–50 mg g^{-1}, see Table 2) was approximately twice as much as that in the flask cultures (23.4 ± 0.9 mg g^{-1} of DCW, Supplementary Table 1).

Nannochloropsis sp. has been described as a “robust industrial alga” and various species/strains of this genus are studied as model strains for biofuel production [38]. High biomass concentrations (up to 10 g DCW L^{-1}) have been reported for outdoor flat-panel photobioreactor cultures [39]. However, we could not reproduce the results for the biomass and EPA production with the two *N. oculata* strains examined in this study. The photobioreactor cultures of *N. oculata* CS-179 showed good growth for the first 5–7 days, followed by sharp declines in biomass concentrations (Fig. 2e). Similar observation was also made with *N. oculata* CS-192 but with larger variations among the biological replicates (Supplementary Fig. 1). Analyses of the nitrate concentration showed that only ~40% of nitrate in the medium had been consumed, indicating that the declines were unlikely to be caused by nutrient limitation. In the photobioreactors, the specific EPA concentration of *N. oculata* CS-179 were also found to be lower than the findings for the flask cultures as well as the values reported in the literature [40] (Fig. 3e and Table 2).

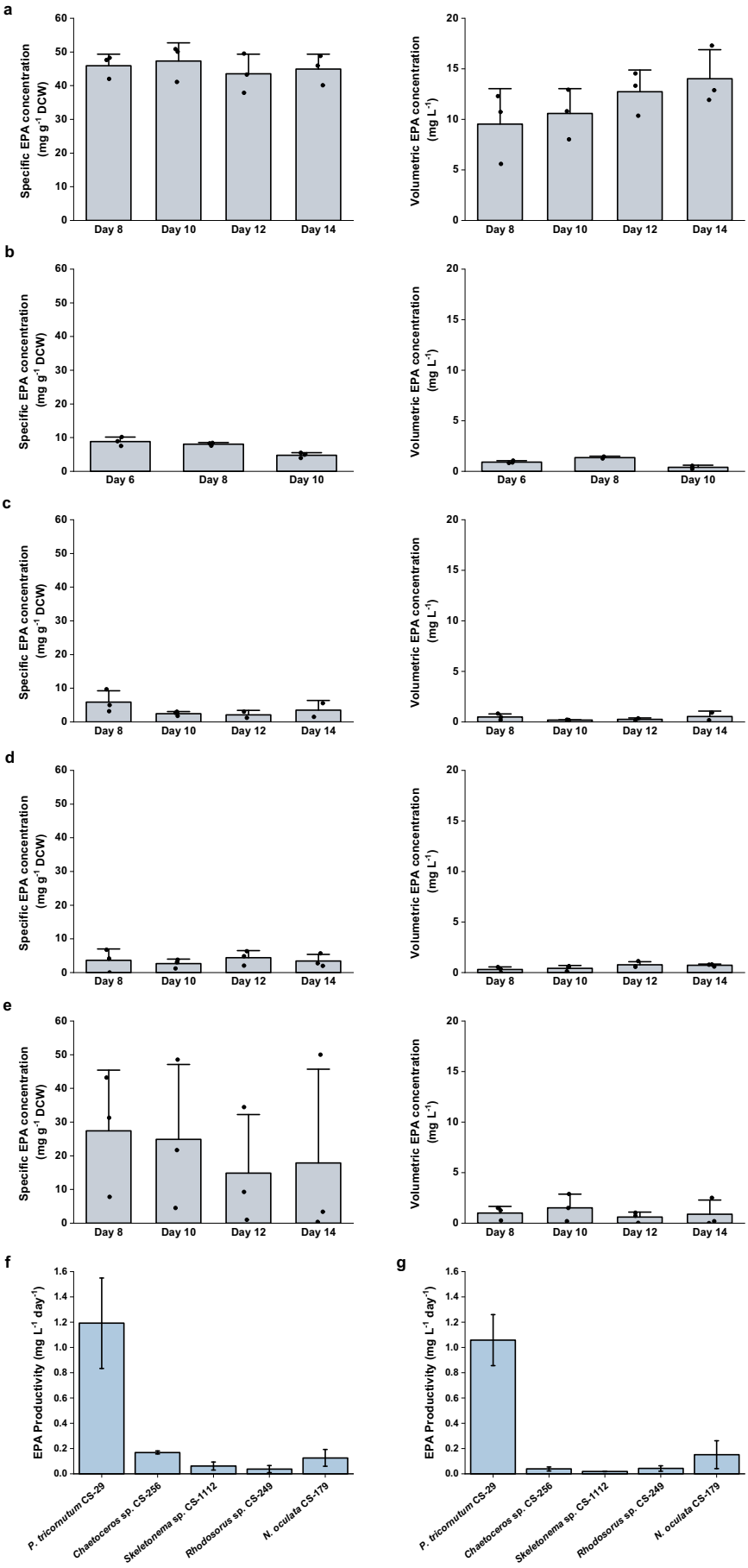

Fig. 3. EPA contents in photobioreactors. (a–e), Specific concentration of EPA per gram of microalgal dry cell weight (DCW) and volumetric concentration of EPA over culture time in the 5-L photobioreactors for microalgal strains *Phaeodactylum tricornutum* CS-29 (a), *Chaetoceros* sp. CS-256. (b), *Skeletonema* sp. CS-1112 (c), *Rhodosorus* sp. CS-249 (d), and *Nannochloropsis oculata* CS-179 (e). (f–g), Comparisons of the five microalgal strains on Day 8 (f) and Day 10 (g) for their EPA productivity. Bar charts represent the mean of the three biological replicates with error bars denoting one standard deviation about the mean (a–g). Specific EPA concentration and EPA productivity of *Chaetoceros* sp. CS-256 on Day 10 and *Skeletonema* sp. 1112 on Days 12 and 14 were calculated with two replicates because the third replicate did not contain sufficient biomass for measurement (b, c, g).

Table 2
Fatty acid composition (% of total fatty acids) and specific concentrations of total fatty acids (TFA) and EPA (mg g^{-1} DCW) of *Phaeodactylum tricornutum* CS-29, *Skeletonema* sp. CS-1112, *Rhodosorus* sp. CS-249, *Nannochloropsis oculata* CS-179 and *Chaetoceros* sp. CS-256 grown in photobioreactors under their standard growth conditions. Values are the mean ± standard deviation ($n = 3$).

Phaeodactylum tricornutum CS-29	**Fatty acid (%TFA)**	**Day 8**	**Day 10**	**Day 12**	**Day 14**
	C14:0	6.5 ± 0.4	6.2 ± 0.2	6.0 ± 0.3	6.0 ± 0.4
	C16:0	21.2 ± 5.5	23.3 ± 6.3	28.8 ± 1.9	29.0 ± 1.6
	C16:1n7	32.0 ± 9.2	33.6 ± 10.2	40.7 ± 1.6	41.5 ± 1.5
	C18:0	0.7 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
	C18:1n9	2.5 ± 0.5	3.3 ± 1.6	5.2 ± 2.2	5.8 ± 1.7
	C18:2n6	1.8 ± 0.3	1.5 ± 0.5	1.0 ± 0.2	0.8 ± 0.1
	C18:3n3	0.6 ± 0.5	0.6 ± 0.4	0.7 ± 0.1	0.7 ± 0.1
	C20:4n6	–	–	–	–
	C20:5n3 (EPA)	32.6 ± 13.7	28.9 ± 15.7	15.7 ± 1.2	14.6 ± 1.1
	C24:0	2.2 ± 0.8	1.9 ± 1.0	1.0 ± 0.1	0.8 ± 0.1
	Specific concentration (mg g^{-1} of DCW)				
	TFA	164.8 ± 58	226± 45	276 ± 12	308 ± 21
	EPA	45.9 ± 2.8	47.4 ± 4.4	43.5 ± 4.8	44.9 ± 3.6
Skeletonema sp. CS-1112	**Fatty acid (%TFA)**	**Day 8**	**Day 10**	**Day 12**	**Day 14**
	C14:0	23.92 ± 15.2	24.2 ± 9.3	33.5 ± 5.2	36.7 ± 5.9
	C16:0	13.3 ± 3.7	14.4 ± 5.9	15.7 ± 1.5	17.5 ± 3.8
	C16:1n7	30.0 ± 6.8	32.3 ± 14.6	34.4 ± 8.1	32.1 ± 7.5
	C18:0	3.5 ± 2.5	2.6 ± 0.5	2.7 ± 0.5	2.7 ± 0.6
	C18:1n9	13.0 ± 4.8	11.9 ± 5.5	5.1 ± 1.9	3.2 ± 0.5
	C18:2n6	4.3 ± 1.8	3.6 ± 1.6	2.5 ± 0.6	2.0 ± 0.2
	C18:3n3	–	–	–	–
	C20:4n6	–	–	–	–
	C20:5n3 (EPA)	12.0 ± 4.2	8.1 ± 3.7	6.1 ± 3.4	5.87 ± 3.1
	C24:0	–	–	–	–
	Specific concentration (mg g^{-1} of DCW)				
	TFA	56.0 ± 26.0	37.0 ± 18.4	45.8 ± 17.2	57.7 ± 4.1
	EPA	5.9 ± 2.8	2.5 ± 0.5	3.1 ± 1.7	3.5 ± 2.0
Rhodosorus sp. CS-249	**Fatty acid (%TFA)**	**Day 8**	**Day 10**	**Day 12**	**Day 14**
	C14:0	–	–	–	–
	C16:0	32.8 ± 15.1	33.5 ± 6.3	31.3 ± 1.5	32.4 ± 3.7
	C16:1n7	2.8 ± 2.1	2.0 ± 1.7	1.2 ± 0.8	0.9 ± 0.6
	C18:0	–	–	–	–
	C18:1n9	13.7 ± 6.8	17.3 ± 1.7	18.3 ± 3.0	21.1 ± 2.6
	C18:2n6	14.7 ± 5.6	10.2 ± 1.4	10.0 ± 1.1	9.7 ± 0.4
	C18:3n3	25.2 ± 17.8	26.2 ± 4.6	25.7 ± 3.1	22.3 ± 5.2
	C20:4n6	3.3 ± 2.6	4.6 ± 1.3	3.9 ± 0.7	3.6 ± 1.2
	C20:5n3 (EPA)	7.4 ± 5.3	6.2 ± 2.0	9.6 ± 2.1	10.0 ± 2.6
	C24:0	–	–	–	–
	Specific concentration (mg g^{-1} of DCW)				
	TFA	38.3 ± 15.4	41.2 ± 5.9	43.9 ± 11.2	32.8 ± 6.8
	EPA	3.6 ± 2.8	2.65 ± 1.1	4.4 ± 1.8	3.5 ± 1.6
Nannochloropsis oculata CS-179	**Fatty acid (%TFA)**	**Day 8**	**Day 10**	**Day 12**	**Day 14**
	C14:0	4.7 ± 0.9	4.8 ± 0.4	5.3 ± 0.1	5.5 ± 0.9
	C16:0	21.1 ± 4.8	24.4 ± 6.4	27.7 ± 6.6	30.8 ± 9.9
	C16:1n7	28.7 ± 3.8	29.3 ± 4.3	34.8 ± 8.8	33.3 ± 4.6
	C18:0	–	–	–	1.4 ± 2.0
	C18:1n9	3.4 ± 0.8	5.2 ± 2.5	6.3 ± 2.5	7.0 ± 2.4
	C18:2n6	2.4 ± 0.3	2.6 ± 0.5	2.1 ± 1.6	1.9 ± 1.6
	C18:3n3	–	–	–	–
	C20:4n6	–	–	–	–
	C20:5n3 (EPA)	39.7 ± 8.2	33.1 ± 11.9	25.6 ± 12.7	20.2 ± 15.3
	C24:0	–	–	–	–
	Specific concentration (mg g^{-1} of DCW)				
	TFA	63.6 ± 26.5	65.6 ± 32.9	43.8 ± 29.7	51.3 ± 49.6
	EPA	27.4 ± 14.7	24.9 ± 18.1	14.2 ± 17.9	17.9 ± 22.7
Chaetoceros sp. CS-256	**Fatty acid (%TFA)**	**Day 6**	**Day 8**	**Day 10**	
	C14:0	22.5 ± 3.9	16.9 ± 3.4	15.5 ± 1.2	
	C16:0	27.8 ± 2.6	29.6 ± 0.5	33.0 ± 3.6	
	C16:1n7	43.5 ± 2.3	45.8 ± 4.6	44.8 ± 4.7	
	C18:0	–	–	–	
	C18:1n9	1.4 ± 0.6	2.4 ± 0.7	3.0 ± 1.2	
	C18:2n6	1.0 ± 0.2	0.8 ± 0.3	0.8 ± 0.3	
	C18:3n3	–	–	–	
	C20:4n6	–	–	–	
	C20:5n3 (EPA)	3.8 ± 0.8	4.4 ± 0.4	3.0 ± 0.2	
	C24:0	–	–	–	
	Specific concentration (mg g^{-1} of DCW)				
	TFA	243.7 ± 51.4	183.7 ± 22.3	158.2 ± 26.9	
	EPA	8.9 ± 1.1	8.1 ± 0.4	4.7 ± 0.6	

(*continued on next page*)

Table 2 (*continued*)

Here it must be noted that the purpose of these experiments was to determine whether results from small-scale screening could be replicated at a scale more representative of industrial photobioreactors. Hence, no attempt was made to optimise the growth conditions at the 5 L scale, and as noted elsewhere [41–45] it is possible to achieve substantial increases in both biomass concentrations and EPA productivity.

Overall, *P. tricornutum* displayed the highest EPA productivities, approximately an order of magnitude higher than those of the other four strains (Fig. 3f–g). Collectively, the photobioreactor screening process indicated that *P. tricornutum* is potentially an excellent candidate for

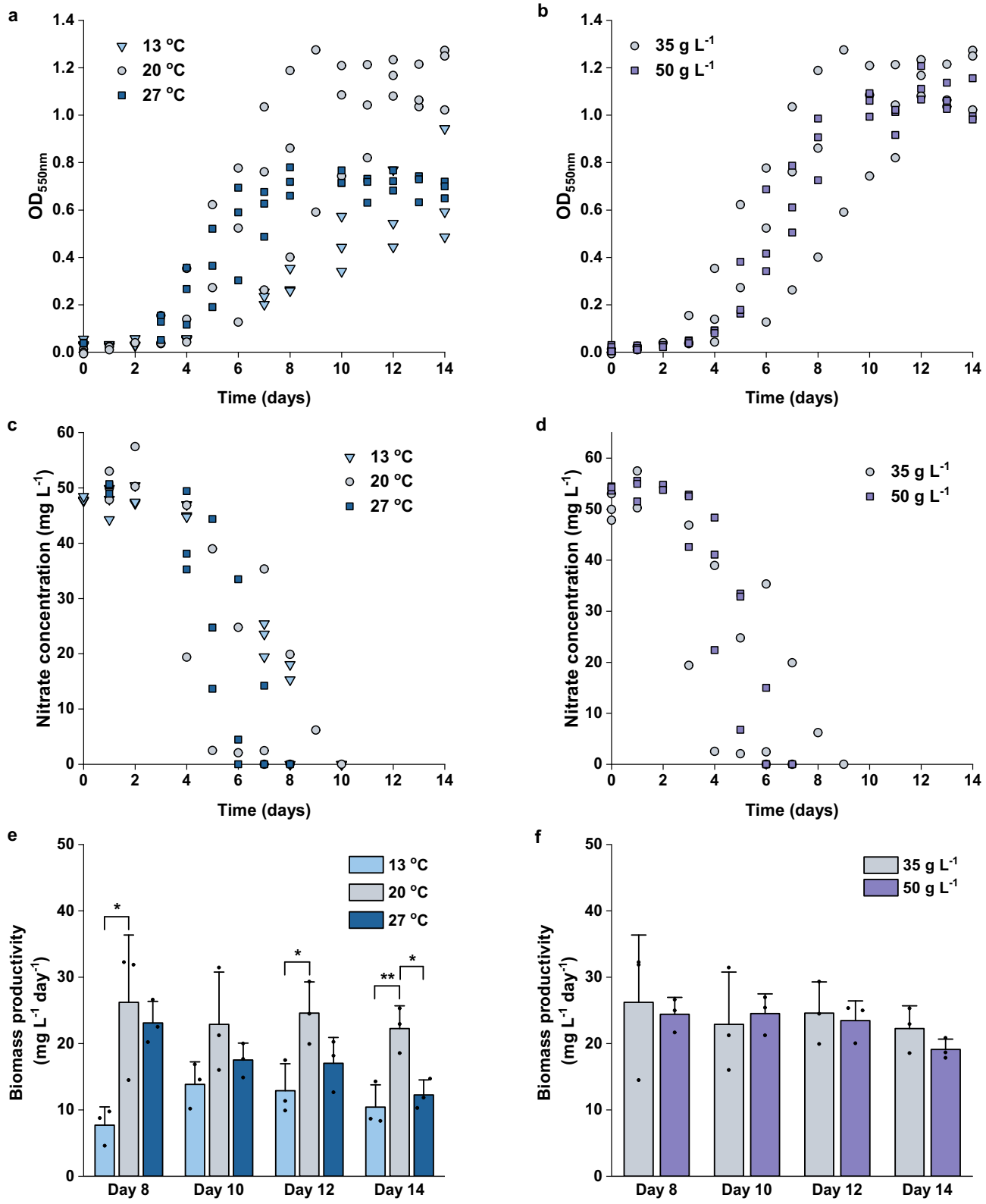

Fig. 4. Effect of temperature and salinity on the growth of *Phaeodactylum tricornutum* CS-29 in photobioreactors. Changes in optical density at 550 nm (a), nitrate concentration (c) and biomass productivity (e) as a function of time for *P. tricornutum* grown in 5-L flat-panel photobioreactors at 13 °C, 20 °C, and 27 °C. Changes in optical density at 550 nm (b), nitrate concentration (d) and biomass productivity (f) of *P. tricornutum* grown in 5-L flat-panel photobioreactors at salt concentrations of 35 and 50 g L^{-1}. All experiments were performed in biologically independent triplicates. Scatter plots represent all replicates (a–d). Bar charts represent the mean of the three biological replicates with error bars denoting one standard deviation about the mean (e, f). One-way ANOVA with Tukey's multiple comparison was performed to compare the biomass productivities at different temperatures on the same day; Day 8: $^{*}P = 0.0274$, Day 12: $^{*}P = 0.0342$, Day 14: $^{**}P = 0.0076$, $^{*}P = 0.0166$ (e). A two-tailed Student's *t*-test was performed to compare the biomass productivities at different salinities on the same day; no significant difference was observed (f).

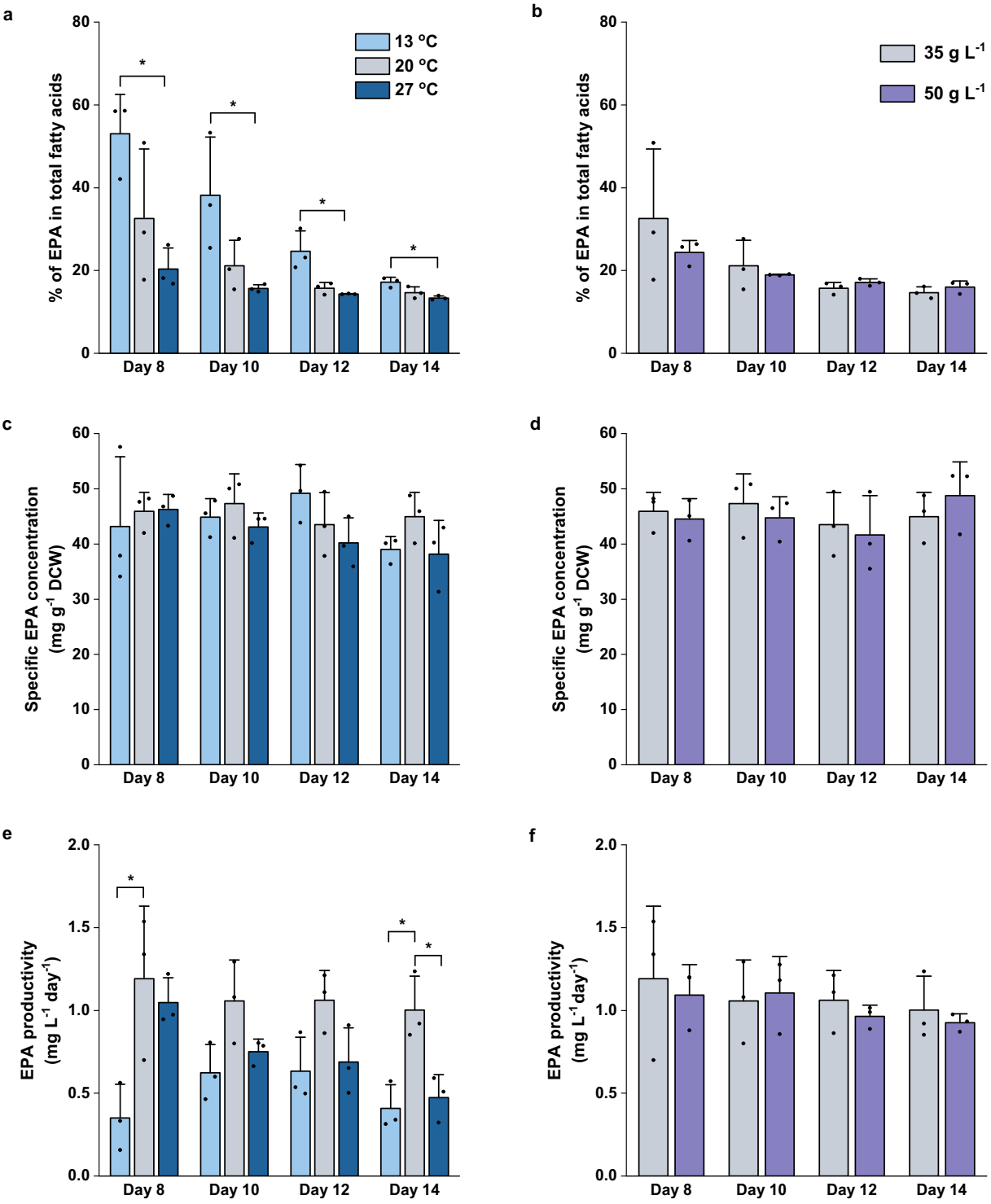

Fig. 5. Effect of temperature and salinity on the EPA production of *Phaeodactylum tricornutum* CS-29 in photobioreactors. Percentage of EPA as a fraction of total fatty acids (a), specific EPA concentration per microalgal dry cell weight (DCW) (c) and EPA productivities (e) of *P. tricornutum* grown in 5-L flat-panel photobioreactors at 13 °C, 20 °C, and 27 °C. Percentage of EPA as a fraction of total fatty acids (b), specific EPA per microalgal DCW (d) and EPA productivity (f) of *P. tricornutum* grown in 5-L flat-panel photobioreactors at salt concentrations of 35 and 50 g L^{-1}. Bar charts represent the mean of the three biological replicates with error bars denoting one standard deviation about the mean (a–f). One-way ANOVA with Tukey's multiple comparison was performed to compare groups at different temperatures; Day 8: $^{*}P = 0.0305$, Day 10: $^{*}P = 0.048$, Day 12: $^{*}P = 0.0214$, Day 14: $^{*}P = 0.0131$ (a); Day 8: $^{*}P = 0.0284$, Day 14: 20°C > 13 °C $^{*}P = 0.0104$, 20 °C > 27 °C $^{*}P = 0.0179$ (e). A two-tailed Student's *t*-test was performed to compare groups at different salinities on the same day; no significant difference was observed.

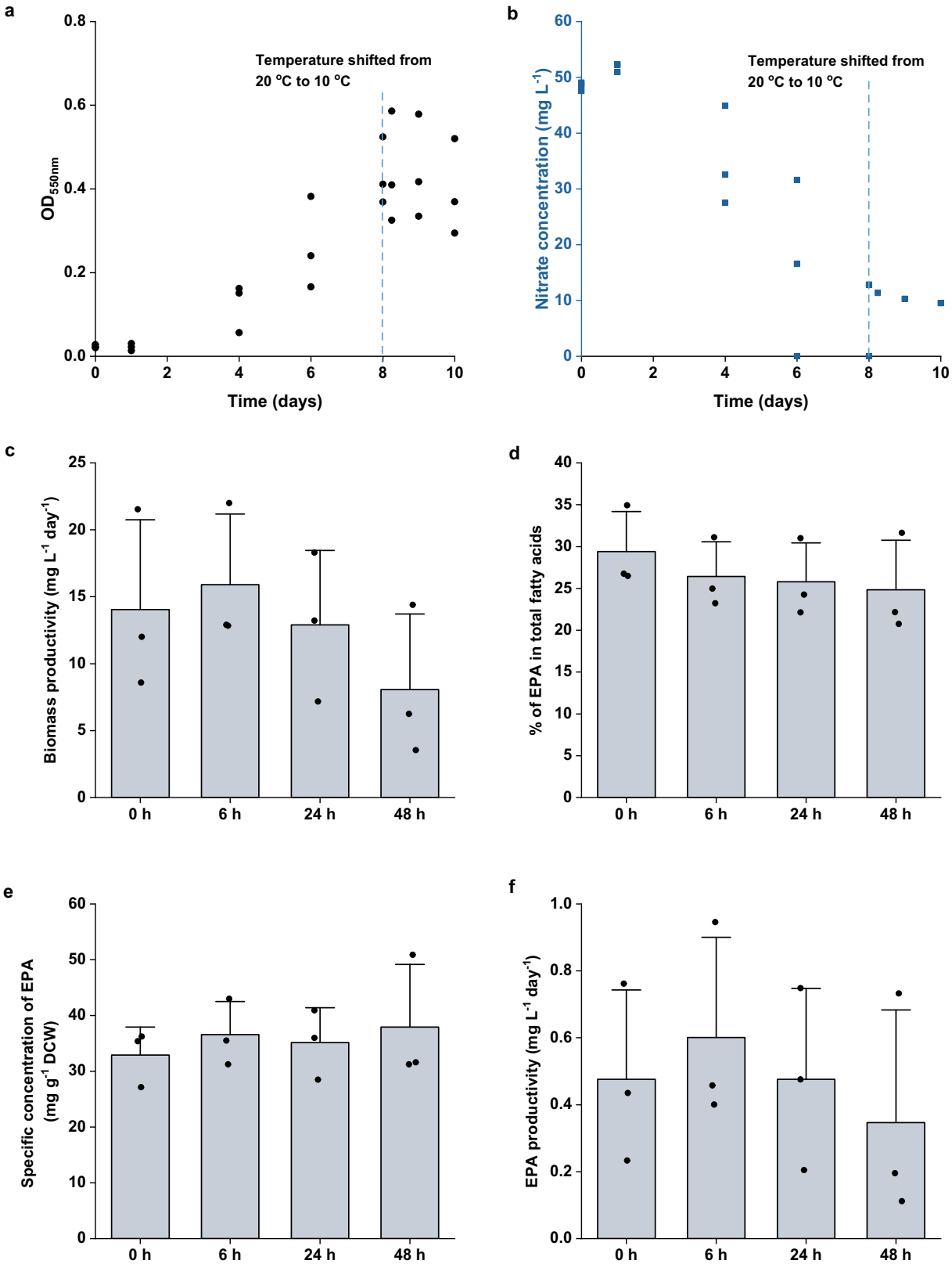

Fig. 6. Effect of temperature downshift on *Phaeodactylum tricornutum* CS-29 in photobioreactors. Changes in optical density at 550 nm (a), nitrate concentration (b), biomass productivity (c), percentage of EPA as a fraction of total fatty acids (d), specific EPA concentration in microalgal dry cell weight (DCW) (e) and EPA productivity (f) 0 h, 6 h, 24 h and 48 h after the growth temperature was shifted from 20 °C to 10 °C. Bar charts represent the mean of the three biological replicates with error bars denoting one standard deviation about the mean (c–f). Statistical significance was calculated using one-way ANOVA with Tukey's multiple comparison; no statistically significant difference was observed ($p \leq 0.05$) (c–f).

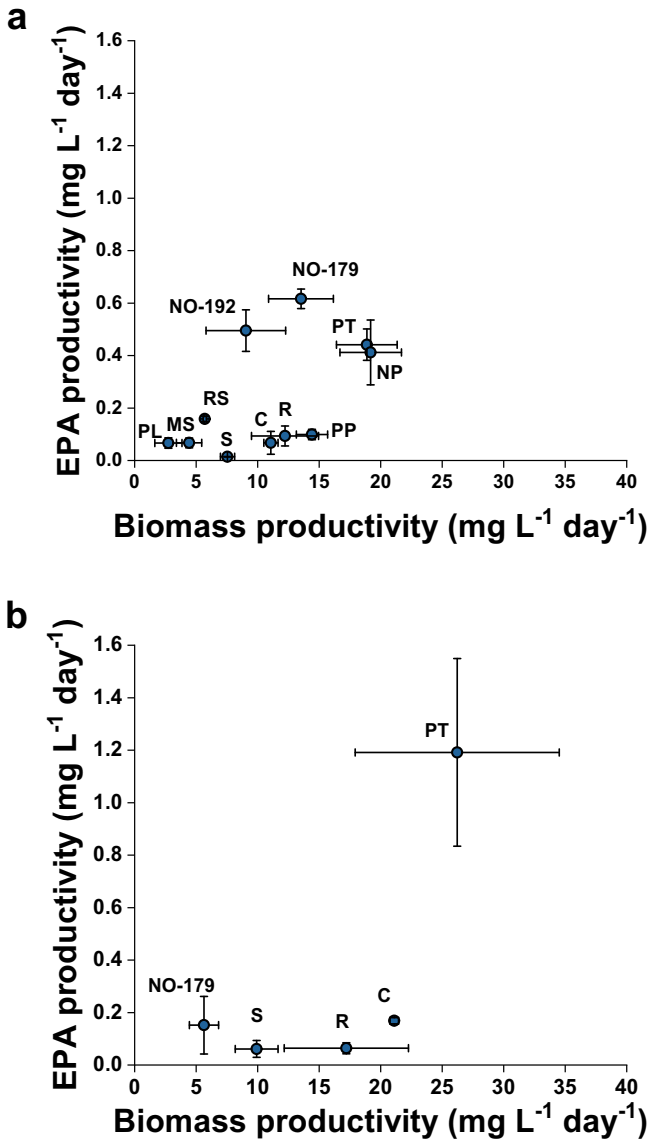

Fig. 7. Comparison of biomass and EPA productivities. (a), Biomass and EPA productivities of the 11 microalgal strains grown in flask cultures. (b), Maximum biomass and EPA productivities of the five microalgal strains successfully grown in 5-L flat-panel photobioreactors. Values represent the mean of three biological replicates with error bars denoting one standard deviation about the mean (a, b). PT: *Phaeodactylum tricornutum* CS-29; NP: *Nitzschia paleacea* CS-430; C: *Chaetoceros* sp. CS-256; S: *Skeletonema* sp. CS-1112; NO-179: *Nannchloropsis oculata* CS-179; NO-192 *Nannochloropsis oculata* CS-192; MS: *Microchloropsis oculata* CS-190; PL: *Pavlova lutheri* CS-182; RS: *Rebecca salina* CS-49; PP: *Porphyridium purpureum* CS-25; R: *Rhodosorus* sp. CS-249. A two-tailed Student's *t*-test was performed to compare the EPA productivities of the flask and photobioreactor cultures for the same strain; PT: photobioreactor > flask $^{*}P = 0.0429$, NO-179: flask > photobioreactor $^{**}P = 0.00479$.

large-scale production of microalgal EPA.

3.3. Effects of temperature and salinity on P. tricornutum in photobioreactors

To investigate how growth temperature could affect the EPA production of *P. tricornutum*, cultures were grown at 13, 20, 27 and 30 °C. *P. tricornutum* was observed to grow in photobioreactors at temperatures between 13 and 27 °C. No growth was observed at 30 °C, consistent with a conclusion reported elsewhere for flask cultures [46,47]. At 13 °C the biomass productivity (7.7 ± 2.2 mg L^{-1} day^{-1}) was ~70% lower than those achieved at 20 and 27 °C (26.2 ± 8.3 and 23.1 ± 2.7 mg L^{-1} day^{-1}, respectively) (Fig. 4e). At 27 °C, the algae entered the stationary phase immediately after the external nitrogen was depleted (Fig. 4a, c). Such results suggest that a temperature of 27 °C may induce heat stress for *P. tricornutum*.

Microorganisms have been shown to increase the degree of fatty acid unsaturation in response to low temperature as a mechanism of maintaining membrane fluidity [48]. We observed a reduction in the percentage of EPA in the fatty acid profile of *P. tricornutum* as the temperature increased, with the percentage of EPA as a fraction of total fatty acids at 13 °C (53% to 17% on Days 8–14) significantly higher than that at 27 °C (20% to 13% on Days 8–14) (Fig. 5a and Supplementary Table 2). However, the specific EPA concentration was not affected by the temperature and remained at 40–50 mg g^{-1} DCW, and no clear effects of temperature on the EPA productivity were observed (Fig. 5c, e).

To further investigate whether hypothermal stress could improve the EPA production of *P. tricornutum*, we also conducted a cold-shock experiment in which cultures were subjected to a sudden downshift of temperature from 20 to 10 °C. The temperature shift occurred within 1 h, after which the cultures were grown for another 48 h. The cold shock severely inhibited the algal growth, with no significant changes in the fatty acid profiles or EPA contents (Fig. 6 and Supplementary Table 3).

To investigate the effects of hypersalinity on its EPA production, *P. tricornutum* was grown in the photobioreactors using f/2 medium prepared at salinities of 35, 50 and 60 g L^{-1}. Increasing the salinity to 50 g L^{-1} had no significant impact on the growth and EPA production of *P. tricornutum* (Figs. 4b, d, f and 5b, d, f and Supplementary Table 4). However, at 60 g L^{-1}, the growth of *P. tricornutum* in photobioreactors showed inconsistency, with great variation among the three biological replicates (Supplementary Fig. 2).

Due to its ability to tolerate a wide range of temperatures *P. tricornutum* is likely to be a good candidate for large-scale, outdoor culture. Similarly, its ability to grow well and produce relatively high amounts of EPA at salt concentrations of 50 g L^{-1} could be advantageous in terms of reducing contamination by less halotolerant species. However, further work at large-scale is necessary to confirm this hypothesis. From a scale-up perspective the robustness of *P. tricornutum* is a clear advantage and further work should focus on improving the biomass productivity.

3.4. Comparison of screening methods

Process development for large-scale production of microalgae-based products starts with strain screening and selection. While the percentage of EPA as a fraction of total fatty acids is a widely used performance parameter in the literature to compare different strains, this parameter alone is a poor guide to strain selection for large-scale cultivation. An algal strain could have a low specific EPA concentration even though its fatty acid profile is rich in EPA, due to a low TFA concentration, as in the cases of the red algae *Rhodosorus* sp. and *P. purpuruem* in this study. Finally, it is necessary to consider other factors when selecting a strain for scale-up [49]. Previous screening studies for identifying EPA-producing strains were often carried out in flask and well-plate cultures under well-regulated conditions [12,14,15,50–52]. Good growth in these small-scale laboratory systems cannot necessarily be translated to large-scale systems. An illustration of this point is that while it was relatively easy to grow all eleven strains examined in flask cultures, only five were able to be grown reproducibly in photobioreactors (and this necessitated modifications to the method in some cases). Of the strains capable of growing in PBRs most showed an improvement in the biomass productivity, as would be expected (Fig. 7). Only *P. tricornutum* was observed to show considerable improvement (an ~3-fold increase) in the EPA productivity.

Small-scale culture methods like flasks and well-plates have advantages of relatively high-throughput, low cost and ease of set-up. Their

Table 3
Nutritional information of *P. tricornutum*.

Proximates (amount per 100 g dry biomass)	
Energy	1682 kJ
Ash	14.6 g
Carbohydrates	28.8 g
Total sugars	3.9 g
Dietary fibre	9.4 g
Total fat	11.6 g
Monounsaturated fat	3.9 g
Polyunsaturated fat	4.7 g
Saturated fat	3.0 g
Trans fat	–
Protein	45.1 g

Amino acid profile (amount per g dry biomass)	
Histidine	8.2 mg
Serine	18.0 mg
Arginine	21.5 mg
Glycine	21.5 mg
Asparagine + aspartic acid	47.2 mg
Glutamine + glutamic acid	60.1 mg
Threonine	19.3 mg
Alanine	26.1 mg
Profline	27.9 mg
Lysine	15.5 mg
Tyrosine	15.0 mg
Methionine	7.3 mg
Valine	18.9 mg
Isoleucine	16.7 mg
Phenylalanine	21.0 mg
Leucine	30.9 mg
Tryptophan	6.9 mg

Minerals (amount per 100 g dry biomass)	
Calcium	150 mg
Sodium	1674 mg
Iron	51.5 mg
Magnesium	472 mg
Potassium	339 mg
Zinc	4.3 mg

Vitamins (amount per 100 g dry biomass)	
Thiamine (vitamin B_1)	1.4 mg
Riboflavin (vitamin B_2)	1.3 mg
Biotin (vitamin B_7)	60 μg
Cobalamin (vitamin B_{12})	14 μg
β-Carotene	3.3 mg

"–": below the detectable level.

major limitation is that they fail to capture many relevant factors for scale-up. For example, species which require attached growth (or form clumps or films) will grow well in flasks. However, their performance in a photobioreactor may be poor due to their inability to grow well in suspension. Similarly, small-scale systems have well-regulated conditions, with very little hydrodynamic stress and few fluctuations in conditions, unlike large-scale photobioreactors where cells experience fluctuations in light intensity (due to mixing), as well as variations in conditions (e.g. temperature). The growth conditions in the 5 L photobioreactors are more representative of those found in large-scale reactors, and hence species capable of reproducibly producing high EPA contents at this scale are likely to be good candidates for further investigation. Similarly, the diameter of the 50 L column used in this work (0.19 m) is very similar to the optimum diameter proposed in the literature [53] for bubble column photobioreactors (0.2 m), meaning results from this scale are likely to be representative of practical photobioreactors.

It is clear that performing strain screening in flask or well-plate cultures is likely to yield misleading results in terms of process scale-up. However, screening a large number of strains in industrial systems or even lab-scale systems, such as the photobioreactors used in this study, is laborious and expensive. This challenge is amplified by the need to perform replicate experiments at multiple conditions in order to better understand its potential performance at industrial-scale.

This calls for a more representative, high-throughput small-scale screening system that can investigate the key operating parameters relevant to large-scale systems, including cell density, hydrodynamic stress, fluctuations in temperature and salinity, light conditions (e.g. incident light intensity and the extent of light attenuation within the culture), the presence of invasive and competing species and inhomogeneities in dissolved O_2 and CO_2 [54]. Good examples of such a system are the scale-down simulators designed for heterotrophic cultures [55]. Such systems should be used to quantify additional performance parameters including biomass and EPA productivities, EPA titre, cell viability and performance consistency.

3.5. Nutritional analysis

Results from the detailed nutritional analysis of the *P. tricornutum* biomass are presented in Table 3. It was found that the dry biomass

contained 45% protein, this being in line with values (31–45%) reported by others [37,56,57] for the same species grown under nitrogen replete conditions. Protein concentrations reported here are also comparable to those found in commercial *Chlorella* and *Spirulina* products (~50%) [58]. In addition to the protein content, it is also important to understand the amino acid profile of the biomass, particularly the profile of essential amino acids. This was quantified by calculating the essential amino acid index (EAAI) using the method outlined by Oser [59] and reference values for human nutrition provided elsewhere [60]. The calculated EAAI was 1.3, with the biomass being particularly rich in aromatic amino acids (i.e. tryptophan, phenylalanine and tyrosine), while being deficient in lysine, methionine and cysteine. Reported [58] average EAAI values for *Chlorella* and *Spirulina* were 1.05 and 1.25 respectively, illustrating that *P. tricornutum* is comparable to these species as a source of protein and amino acids. In addition to the concentration of nutrients it is also important to understand their bioavailability. Neumann et al. [61] examined this issue, finding that addition of up to 25% *P. tricornutum* biomass to the diet had no negative effects in mice, and that the protein availability was similar to that of the control diet.

As well as being a source of macronutrients (i.e. lipids and protein), the algal biomass also contains micronutrients like vitamins and minerals. Vitamin concentrations reported in Table 3 are lower than those reported [62] in the literature for *Nannochloropsis*. Similarly, mineral concentrations were generally lower [56] than those previously reported for *P. tricornutum*, however this difference may be attributed to differences in the growth medium and biomass processing steps used.

Based on the results presented in Table 3, a serving of 5–6 g of dry *P. tricornutum* biomass is sufficient to meet the daily EPA requirement for adults [63]. In comparison, approximately 1.2–1.4 g of fish oil, or 12–14 g of cooked fish are necessary to meet the adequate intake for EPA and DHA (values were calculated using data from the USDA database [64], assuming 100% bioavailability). This comparison suggests that whole algal biomass could feasibly be incorporated into the diet as a source of EPA. In addition to being a source of EPA, a 5–6 g serving of dry *P. tricornutum* biomass also provides approximately 15% and 30% of the recommended intakes of iron and vitamin B_{12}, respectively [65]. Such results are promising and demonstrate the need to further understand the digestibility of algal biomass, its potential to be formulated into food products and the health impacts of such products.

4. Conclusions

A key factor in successful process scale-up is the screening and selection of an appropriate species. Extensive work has been done using small-scale culture systems to quantify the fatty acid composition of many species of microalgae, and this work aimed to determine whether such an approach could be used to guide process scale-up. It was found that it was challenging to extrapolate results from flask cultures to cultures grown in 5 L photo-bioreactors. For example, all eleven species examined grew easily in flasks, while only five grew reproducibly in photo-bioreactors. It is thought that this difference is due to the inability of some species to grow in suspension, tolerate hydrodynamic stress or exposure to fluctuating light intensities. This suggests there is a need for improved, small-scale screening systems which better replicate the conditions found in larger reactors.

Of the species examined *P. tricornutum* was found to display the best performance in the 5 L bioreactors used. It was found that it had a relatively high EPA content (~5% of DCW), as well as the ability to tolerate a range of temperatures (13–27 °C) and moderate hypersalinity (up to 50 g L^{-1}). Additionally, it was also found that the specific EPA content remained constant for the conditions examined. The ability to tolerate a range of conditions, while maintaining a consistent EPA content of ~5% of DCW is obviously favourable from a scale-up perspective. Finally, the composition of the biomass was characterized, in addition to being a good source of EPA it was found to be rich in protein (45% DCW) and vitamin B_{12}. Future work should examine ways to further scale-up the process and improve the biomass productivity. Additionally, research should investigate the formulation of *P. tricornutum* biomass into foods and quantify the bioavailability and health benefits of algae in the diet.

Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this study.

CRediT authorship contribution statement

Wenjia Gu: Conceptualization, Investigation, Writing (Original Draft and Review & Editing), Visualization.

John Kavanagh: Conceptualization, Resources, Supervision and Writing (Review & Editing).

Dale McClure: Conceptualization, Methodology, Investigation, Writing (Review & Editing), Resources, and Supervision.

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.

Acknowledgements

The authors would like to acknowledge the Australian Government for its support through the provision of an Australian Government Research Training Program Scholarship.

The authors would like to acknowledge Mr. Toomas Mirlieb, from the School of Chemical and Biomolecular Engineering, the University of Sydney, for conducting the initial literature review for this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2021.102564.

References

[1] R.K. Saini, Y.-S. Keum, Omega-3 and omega-6 polyunsaturated fatty acids: dietary sources, metabolism, and significance—a review, Life Sci. 203 (2018) 255–267.
[2] A.P. Simopoulos, Omega-3 fatty acids in health and disease and in growth and development, Am. J. Clin. Nutr. 54 (1991) 438–463.
[3] C. Ruxton, S.C. Reed, M. Simpson, K. Millington, The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence, J. Hum. Nutr. Diet. 17 (2004) 449–459.
[4] J. Tur, M. Bibiloni, A. Sureda, A. Pons, Dietary sources of omega 3 fatty acids: public health risks and benefits, Br. J. Nutr. 107 (2012) S23–S52.
[5] T.C. Adarme-Vega, S.R. Thomas-Hall, P.M. Schenk, Towards sustainable sources for omega-3 fatty acids production, Curr. Opin. Biotechnol. 26 (2014) 14–18.
[6] A.P. Bimbo, Current and future sources of raw materials for the long-chain omega-3 fatty acid market, Lipid Technol. 19 (2007) 176–179.
[7] H.A. Hamilton, R. Newton, N.A. Auchterlonie, D.B. Müller, Systems approach to quantify the global omega-3 fatty acid cycle, Nat. Food 1 (2020) 59–62.
[8] D.R. Tocher, Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective, Aquaculture 449 (2015) 94–107.
[9] M.A. Borowitzka, High-value products from microalgae—their development and commercialisation, J. Appl. Phycol. 25 (2013) 743–756.
[10] Z. Xue, P.L. Sharpe, S.-P. Hong, N.S. Yadav, D. Xie, D.R. Short, H.G. Damude, R. A. Rupert, J.E. Seip, J. Wang, D.W. Pollak, M.W. Bostick, M.D. Bosak, D.J. Macool, D.H. Hollerbach, H. Zhang, D.M. Arcilla, S.A. Bledsoe, K. Croker, E.F. McCord, B. D. Tyreus, E.N. Jackson, Q. Zhu, Production of omega-3 eicosapentaenoic acid by metabolic engineering of Yarrowia lipolytica, Nat. Biotechnol. 31 (2013) 734–740.
[11] R.B. Draaisma, R.H. Wijffels, P.E. Slegers, L.B. Brentner, A. Roy, M.J. Barbosa, Food commodities from microalgae, Curr. Opin. Biotechnol. 24 (2013) 169–177.
[12] J. Volkman, S. Jeffrey, P. Nichols, G. Rogers, C. Garland, Fatty acid and lipid composition of 10 species of microalgae used in mariculture, J. Exp. Mar. Biol. Ecol. 128 (1989) 219–240.
[13] W. Yongmanitchai, O.P. Ward, Screening of algae for potential alternative sources of eicosapentaenoic acid, Phytochemistry 30 (1991) 2963–2967.

[14] S.M. Renaud, L.-V. Thinh, D.L. Parry, The gross chemical composition and fatty acid composition of 18 species of tropical Australian microalgae for possible use in mariculture, Aquaculture 170 (1999) 147–159.
[15] V. Patil, T. Källqvist, E. Olsen, G. Vogt, H.R. Gislerød, Fatty acid composition of 12 microalgae for possible use in aquaculture feed, Aquacult. Int. 15 (2007) 1–9.
[16] W. Gu, J.M. Kavanagh, D.D. McClure, Photoautotrophic production of eicosapentaenoic acid, Crit. Rev. Biotechnol. 1–18 (2021).
[17] T.L. da Silva, A. Reis, Scale-up problems for the large scale production of algae, in: Algal Biorefinery: An Integrated Approach, Springer, 2015, pp. 125–149.
[18] X. Ma, L. Zhang, B. Zhu, K. Pan, S. Li, G. Yang, Low-temperature affected LC-PUFA conversion and associated gene transcript level in Nannochloropsis oculata CS-179, J. Ocean Univ. China 10 (2011) 270–274.
[19] H. Tatsuzawa, E. Takizawa, Changes in lipid and fatty acid composition of Pavlova lutheri, Phytochemistry 40 (1995) 397–400.
[20] V. Pasquet, L. Ulmann, V. Mimouni, F. Guihéneuf, B. Jacquette, A. Morant-Manceau, G. Tremblin, Fatty acids profile and temperature in the cultured marine diatom Odontella aurita, J. Appl. Phycol. 26 (2014) 2265–2271.
[21] H. Jiang, K. Gao, Effects of lowering temperature during culture on the production of polyunsaturated fatty acids in the marine diatom *Phaeodactylum tricornutum* (Bacillariophyceae), J. Phycol. 40 (2004) 651–654.
[22] S.H. Mortensen, K.Y. Børsheim, J. Rainuzzo, G. Knutsen, Fatty acid and elemental composition of the marine diatom Chaetoceros gracilis Schütt. Effects of silicate deprivation, temperature and light intensity, J. Exp. Mar. Biol. Ecol. 122 (1988) 173–185.
[23] S.M. Renaud, L.-V. Thinh, G. Lambrinidis, D.L. Parry, Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures, Aquaculture 211 (2002) 195–214.
[24] T. Řezanka, J. Doucha, P. Mareš, M. Podojil, Effect of cultivation temperature and light intensity on fatty acid production in the red alga *Prophyridium cruentum*, J. Basic Microbiol. 27 (1987) 275–278.
[25] S. Fon-Sing, M. Borowitzka, Isolation and screening of euryhaline Tetraselmis spp. suitable for large-scale outdoor culture in hypersaline media for biofuels, J. Appl. Phycol. 28 (2016) 1–14.
[26] L. Zanella, F. Vianello, Microalgae of the genus Nannochloropsis: chemical composition and functional implications for human nutrition, J. Funct. Food. 68 (2020), 103919.
[27] R.F. Jones, H.L. Speer, W. Kury, Studies on the growth of the red alga Porphyridium cruentum, Physiol. Plant. 16 (1963) 636–643.
[28] T.D. Tarento, D.D. McClure, E. Vasiljevski, A. Schindeler, F. Dehghani, J. M. Kavanagh, Microalgae as a source of vitamin K1, Algal Res. 36 (2018) 77–87.
[29] D.D. McClure, J.K. Nightingale, A. Luiz, S. Black, J. Zhu, J.M. Kavanagh, Pilot-scale production of lutein using Chlorella vulgaris, Algal Res. 44 (2019), 101707.
[30] American Public Health Association, 4500-NO3- NITROGEN (NITRATE), in: E.W. B. Rice, B. Rodger, Andrew D. Eaton, Lenore S. Clesceri (Eds.), Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Washington DC, 2012, pp. 4–122.
[31] G. Breuer, W.A. Evers, J.H. de Vree, D.M. Kleinegris, D.E. Martens, R.H. Wijffels, P. P. Lamers, Analysis of fatty acid content and composition in microalgae, J. Vis. Exp. 80 (2013), e50628.
[32] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (1959) 911–917.
[33] E. Ryckebosch, C. Bruneel, R. Termote-Verhalle, K. Goiris, K. Muylaert, I. Foubert, Nutritional evaluation of microalgae oils rich in omega-3 long chain polyunsaturated fatty acids as an alternative for fish oil, Food Chem. 160 (2014) 393–400.
[34] Z. Cohen, C. Ratledge, Searching for Polyunsaturated Fatty Acid-rich Photosynthetic Microalgae, Single Cell Oils - Microbial and Algal Oils, 2nd Edition, AOCS Press, 2010.
[35] M.R. Droop, 25 years of algal growth kinetics a personal view, Bot. Mar. 26 (1983) 99–112.
[36] K.J. Flynn, Use, abuse, misconceptions and insights from quota models—the droop cell quota model 40 years on, Oceanogr. Mar. Biol. Annu. Rev. 46 (2008) 1–23.
[37] O. Levitan, J. Dinamarca, E. Zelzion, D.S. Lun, L.T. Guerra, M.K. Kim, J. Kim, B. A. Van Mooy, D. Bhattacharya, P.G. Falkowski, Remodeling of intermediate metabolism in the diatom Phaeodactylum tricornutum under nitrogen stress, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 412–417.
[38] O. Kilian, C.S. Benemann, K.K. Niyogi, B. Vick, High-efficiency homologous recombination in the oil-producing alga Nannochloropsis sp, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 21265–21269.
[39] N. Zou, A. Richmond, Effect of light-path length in outdoor fiat plate reactors on output rate of cell mass and of EPA in Nannochloropsis sp, Prog. Ind. Microbiol. (Elsevier 1999) 351–356.
[40] C.J. Hulatt, R.H. Wijffels, S. Bolla, V. Kiron, PLOS ONE 12 (2017), e0170440.
[41] N. Zou, A. Richmond, Effect of light-path length in outdoor flat plate reactors on output rate of cell mass and of EPA in Nannochloropsis sp, J. Biotechnol. 70 (1999) 351–356.
[42] J.M.F. Sevilla, M.C. Cerón García, A. Sánchez Mirón, E.H. Belarbi, F.G. Camacho, E. M. Grima, Pilot-plant-scale outdoor mixotrophic cultures of Phaeodactylum tricornutum using glycerol in vertical bubble column and airlift photobioreactors: studies in fed-batch mode, Biotechnol. Prog. 20 (2004) 728–736.
[43] A. Satoh, K. Ichii, M. Matsumoto, C. Kubota, M. Nemoto, M. Tanaka, T. Yoshino, T. Matsunaga, T. Tanaka, A process design and productivity evaluation for oil production by indoor mass cultivation of a marine diatom, Fistulifera sp. JPCC DA0580, Bioresour. Technol. 137 (2013) 132–138.
[44] F.G.A. Fernández, J.A.S. Pérez, J.M.F. Sevilla, F.G. Camacho, E.M. Grima, Modeling of eicosapentaenoic acid (EPA) production from Phaeodactylum tricornutum cultures in tubular photobioreactors. Effects of dilution rate, tube diameter, and solar irradiance, Biotechnol. Bioeng. 68 (2000) 173–183.
[45] B. Gao, A. Chen, W. Zhang, A. Li, C. Zhang, Co-production of lipids, eicosapentaenoic acid, fucoxanthin, and chrysolaminarin by Phaeodactylum tricornutum cultured in a flat-plate photobioreactor under varying nitrogen conditions, J. Ocean Univ. China 16 (2017) 916–924.
[46] J.M. Rousch, S.E. Bingham, M.R. Sommerfeld, Changes in fatty acid profiles of thermo-intolerant and thermo-tolerant marine diatoms during temperature stress, J. Exp. Mar. Biol. Ecol. 295 (2003) 145–156.
[47] E.Bitaubé Pérez, I.Caro Pina, L.Pérez Rodríguez, Kinetic model for growth of Phaeodactylum tricornutum in intensive culture photobioreactor, Biochem. Eng. J. 40 (2008) 520–525.
[48] S.L. Neidleman, Effects of temperature on lipid unsaturation, Biotechnol. Genet. Eng. Rev. 5 (1987) 245–268.
[49] J.D. McMillan, G.T. Beckham, Thinking big: towards ideal strains and processes for large-scale aerobic biofuels production, Microb. Biotechnol. 10 (2017) 40.
[50] P. Steinrücken, S.R. Erga, S.A. Mjøs, H. Kleivdal, S.K. Prestegard, Bioprospecting North Atlantic microalgae with fast growth and high polyunsaturated fatty acid (PUFA) content for microalgae-based technologies, Algal Res. 26 (2017) 392–401.
[51] L. Archer, D. Mc Gee, A. Paskuliakova, G.R. McCoy, T. Smyth, E. Gillespie, N. Touzet, Fatty acid profiling of new irish microalgal isolates producing the high-value metabolites EPA and DHA, Algal Res. 44 (2019), 101671.
[52] G.A. Dunstan, J.K. Volkman, S.M. Barrett, J.-M. Leroi, S. Jeffrey, Essential polyunsaturated fatty acids from 14 species of diatom (Bacillariophyceae), Phytochemistry 35 (1993) 155–161.
[53] A.Sánchez Mirón, A.Contreras Gómez, F. García Camacho, E.Molina Grima, Y. Chisti, Comparative evaluation of compact photobioreactors for large-scale monoculture of microalgae, J. Biotechnol. 70 (1999) 249–270.
[54] F. Delvigne, R. Takors, R. Mudde, W. van Gulik, H. Noorman, Bioprocess scale-up/down as integrative enabling technology: from fluid mechanics to systems biology and beyond, Microb. Biotechnol. 10 (2017) 1267–1274.
[55] F. Li, Y. Hashimura, R. Pendleton, J. Harms, E. Collins, B. Lee, A systematic approach for scale-down model development and characterization of commercial cell culture processes, Biotechnol. Prog. 22 (2006) 696–703.
[56] M.M. Rebolloso-Fuentes, A. Navarro-Pérez, J.J. Ramos-Miras, J.L. Guil-Guerrero, Biomass nutrient profiles of the microalga Phaeodactylum tricornutum, J. Food Biochem. 25 (2001) 57–76.
[57] Y. Cui, S.R. Thomas-Hall, E.T. Chua, P.M. Schenk, Development of a Phaeodactylum tricornutum biorefinery to sustainably produce omega-3 fatty acids and protein, J. Clean. Prod. 300 (2021), 126839.
[58] M. Muys, Y. Sui, B. Schwaiger, C. Lesueur, D. Vandenheuvel, P. Vermeir, S. E. Vlaeminck 275 (2019) 247–257.
[59] B.L. Oser, An integrated essential amino acid index for predicting the biological value of proteins 281 (1959).
[60] F. Joint, W.H. Organization, Protein and Amino Acid Requirements in Human Nutrition: Report of a Joint FAO/WHO/UNU Expert Consultation, World Health Organization, 2007.
[61] U. Neumann, F. Derwenskus, A. Gille, S. Louis, U. Schmid-Staiger, K. Briviba, S. C. Bischoff, Bioavailability and safety of nutrients from the microalgae Chlorella vulgaris, Nannochloropsis oceanica and Phaeodactylum tricornutum in C57BL/6 mice, Nutrients 10 (2018).
[62] M.R. Brown, M. Mular, I. Miller, C. Farmer, C. Trenerry, The vitamin content of microalgae used in aquaculture, J. Appl. Phycol. 11 (1999) 247–255.
[63] Food and Agriculture Organisation of the United Nations, Fats and Fatty Acids in Human Nutrition - Report of an Expert Consultation, 2010. Geneva.
[64] US Department of Agriculture, Nutrient Search, 2020.
[65] World Health Organization, Vitamin and Mineral Requirements in Human Nutrition, World Health Organization, 2004.