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Growth and LC-PUFA production of the cold-adapted microalga *Koliella antarctica* in photobioreactors

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Summary

Aquaculture is a fast-growing industry that plays an increasing role in producing food for humans. Production of quality aquafeeds requires a significant amount of long-chain polyunsaturated fatty acids (LC-PUFAs), that are currently derived from fish oil. However, the dependence of aquafeed production on fish oil can reduce the amount of food available for direct human consumption and may have negative consequences to wild fish stocks by overfishing. The current supply of LC-PUFAs is already insufficient to meet global market demands and the price has dramatically increased in the last few decades. Therefore, there is an urgent need to find alternative aquafeed ingredients which contain essential LC-PUFAs and offer high nutritional values. Microalgae are the primary source of LC-PUFAs in aquatic ecosystems and are considered promising alternative sources of aquafeed ingredients. They can be grown rapidly, often in saline or waster, and can make use of marginal land. Thus, cultivated microalgae could offer much higher areal productivities and offer products with a small ecological footprint. Although the use of microalgae-derived products as aquafeed is very promising, successful industrial algae production is still rare. In particular, commonly used temperate and warm-water microalgae display substantially lower yield, or cannot survive, in cooler climates. Bioprospecting and optimization of strains that are able to grow rapidly at lower temperatures could enable year-round utilization of culture facilities in temperate zones, and promote microalgae cultivation in cooler climates. In this work, the cold-adapted microalga *Koliella antarctica* (Trebouxiophyceae) was cultivated at 15 °C to optimize growth and LC-PUFA production in bubble-tube and flat-plate photobioreactors. The impact of nitrogen starvation, phosphorus starvation, salinity and light intensity on the growth, fatty acid composition and protein content was investigated. After culture optimization, the maximum biomass productivity was 2.37 g L^{-1} d^{-1}, which is comparable to that of temperate strains in similar cultivation systems. Amongst all conditions tested, the maximum total fatty acid (TFA) content measured 271.9 mg g^{-1} dry weight in the late stationary phase. Nitrogen and phosphorus starvation strongly induced neutral lipid (TAG) accumulation, up to 90.3% of TFA, that mostly consisted of the monounsaturated fatty acid C18:1n-9 (oleic acid, OA). PUFAs were also abundant and together accounted for 30-45% of total TAG. The highest

eicosapentaenoic acid (EPA, C20:5n-3) content amounted to 6.7 mg g^{-1} dry weight (4.9% TFA) in control treatments, whilst the highest arachidonic acid (ARA, C20:4n-6) content was 9.6 mg g^{-1} dry weight (3.5% TFA) in the late stationary phase. Phosphorus starvation was the best strategy to obtain high total fatty acid yields (mg L^{-1}) whilst maintaining the protein, total PUFA and long-chain omega-3 fatty acid contents. The strong growth rate of *K. antarctica* coupled with its favorable biochemical composition, including LC-PUFAs, may make this strain suitable for inclusion in aquafeed products. These findings indicate that *K. antarctica* and similar cold-adapted microalgae could be productive cell factories for PUFA and protein production in cooler climates.

General introduction

1.1 Current status of aquaculture

The world's population is increasing by roughly 83 million people every year, and estimated to reach 9.8 billion by 2050 (UN 2017). To meet the nutritional demands for the increasing population, food production needs to increase by 68 % in 2050 compared with 2005/2007 (Alexandratos and Bruinsma 2012). Aquaculture plays an increasing role in providing humans with high-quality protein and lipid sources. Aquaculture food production has drastically increased, whilst capture fishery production has stagnated since the late 1980s (FAO 2016, Fig. A). In 1990, total global aquaculture production was only 13 million tones, whereas in 2014 was 73.8 million tonnes with a value of 160.2 billion USD (FAO 2016). World aquaculture production in 2014 accounted for 44.1% of the total production from fisheries and aquaculture, up from 31.1% in 2004 (FAO 2016). The demand to produce more food in aquaculture is increasing not only due to population growth, but also due to increased consumer wealth in developing countries and preference towards healthy food (FAO 2016). Providing a sustainable source of aquafeed with high-quality ingredients is key for successful growth and the future sustainability of aquaculture (Glencross et al. 2007; Deutsch et al. 2007).

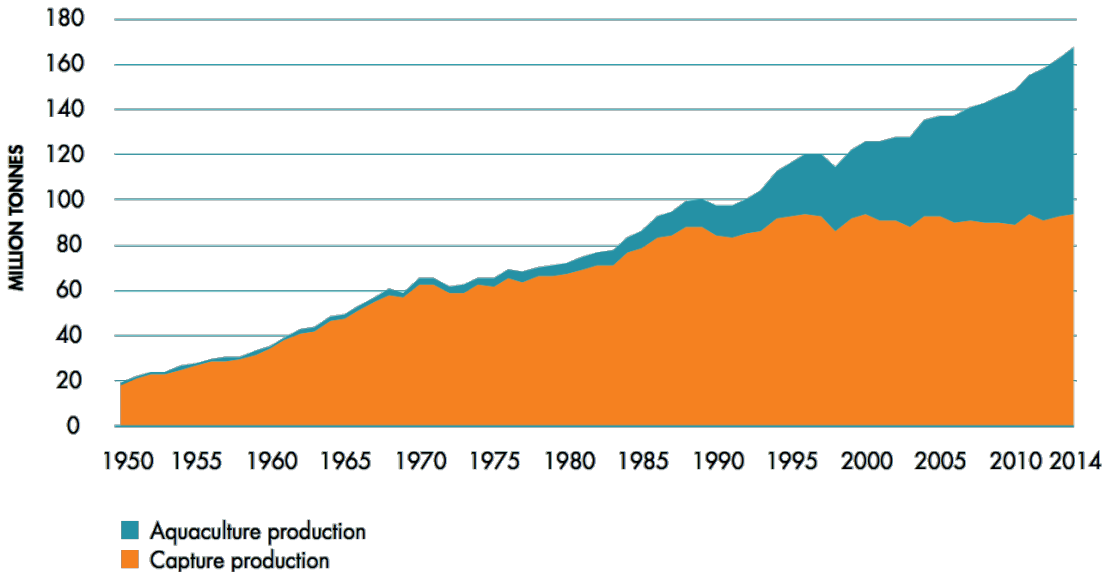

Fig A. World capture fisheries and aquaculture production from 1950 to 2014.

1.2 Aquaculture - need for alternative feed ingredients

Production of aquafeeds traditionally relies on fishmeal and fish oil, which originate from capture fisheries (Tacon and Metian 2008). Fishmeal is considered an ideal raw material due to its high protein content (51-72%), high nutrient digestibility, excellent amino acid profile, and high palatability (Gatlin Delbert et al. 2007). Fish oil is considered the optimal quality oil for farmed aquatic species. It provides long-chain polyunsaturated fatty acids (LC-PUFAs) such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (ARA) that some fish cannot obtain at the required levels by endogenous synthesis (Glencross 2009). However, the dependence of aquafeed production on fishmeal and fish oil has been criticized for reducing the amount of food available for direct human consumption, as well as the consequences to wild fish stocks by overfishing (Deutsch et al. 2007; Naylor et al. 2000). The current supply of fishmeal and fish oil is already insufficient to meet global market demands and the prices of fishmeal and fish oil have shown significant increases in the last few decades (Fig. B, Fig. C). For example, fishmeal prices have risen by more than two-fold in the past ten years (FAO 2016). Therefore, terrestrial plant-based substitutes have been employed to replace conventional fishmeal and fish oil in farmed fish feeds (Hardy Ronald 2010; Turchini et al. 2011).

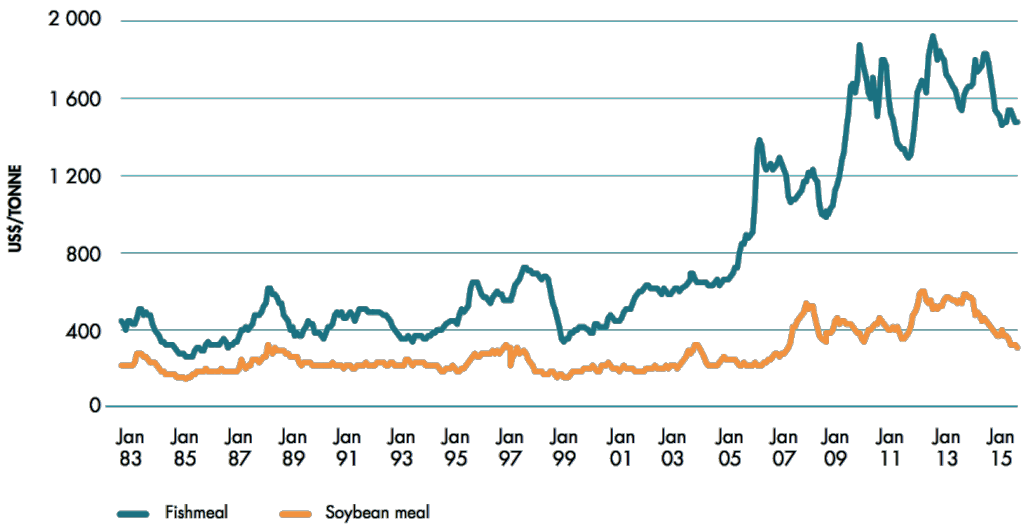

Fig B. Fishmeal and soybean meal prices in Germany and the Netherlands from 1983 to 2015 (FAO 2016).

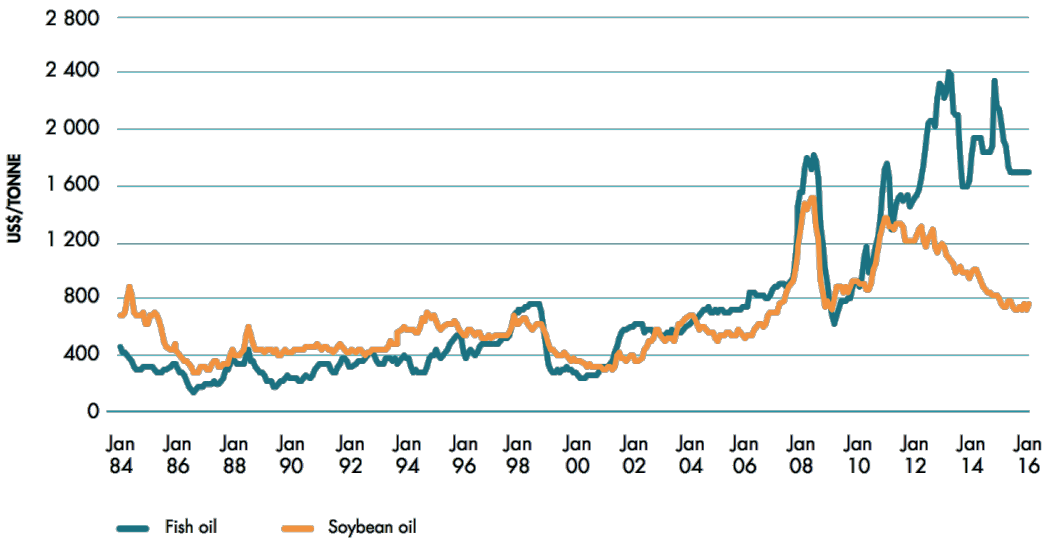

Fig C. Fish oil and soybean oil prices in the Netherlands from 1984 to 2016 (FAO 2016).

Norwegian salmon feed, for example, successfully reduced the dependence on fishmeal and fish oil, and contained 66% plant ingredients in the 2012 diet (Ytrestøyl et al. 2015, Fig. D). These plant ingredients include soy protein concentrate, wheat gluten, fava beans, pea protein, maize gluten, and rapeseed oil (Turchini et al. 2011; Nasopoulou and Zabetakis 2012). However, the use of plant ingredients has drawbacks such as lower protein content, the absence of LC-PUFAs, and the presence of anti-nutritional factors that can cause intestinal inflammation in fish such as Atlantic salmon (Krogdahl et al. 2010; Francis et al. 2001; Carter and Hauler 2000). The intensive replacement of fishmeal and fish oil by plant ingredients has also resulted in high levels of omega-6 fatty acids and low levels of long chain omega-3 fatty acids in fish feeds (Ytrestøyl et al. 2015). This has led to a decline in EPA and DHA levels in final fish products, which may decrease the health benefits for human consumption (Sprague et al. 2016). Therefore, there is an urgent need to find alternative aquafeed ingredients which can provide quality nutritional values.

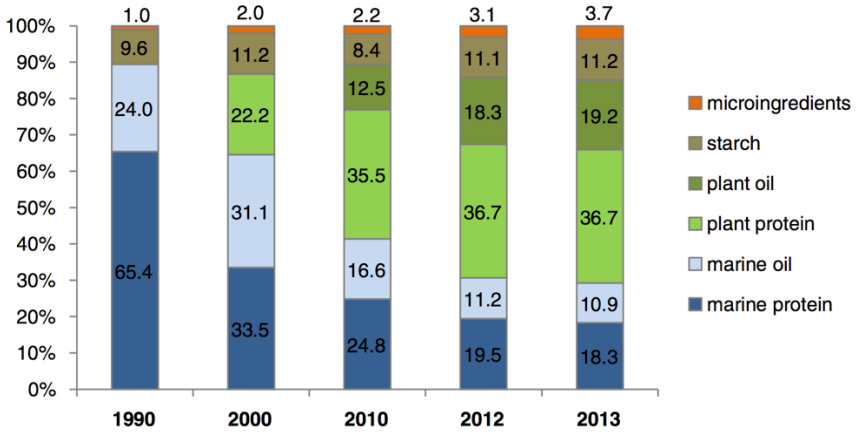

Fig D. Nutrient sources in Norwegian salmon farming from 1990 to 2013. Each ingredient type is shown as its percentage of the total diet (Ytrestøyl et al. 2015).

1.3 Microalgae as an alternative source of aquafeed

Microalgae are the natural diet of many fish and the primary producers of LC-PUFAs in the marine food web (Adarme-Vega et al. 2012). They are considered promising alternative sources for aquafeed because their nutritional value closely resembles fishmeal and fish oil (Khozin-Goldberg et al. 2011; Brown et al. 1997). Microalgae can also synthesize bioactive compounds and essential nutrients for aquatic species such as essential amino acids, essential fatty acids, vitamins, and pigments (Yaakob et al. 2014). Many feeding studies have demonstrated that some microalgae strains have promising features as aquafeed ingredients due to a high protein content, highly digestible protein (Skrede et al. 2011) and the presence of LC-PUFAs (Kousoulaki et al. 2015; Miller et al. 2007; Martins et al. 2013). Microalgae have also been reported to possess additional functional properties that may benefit health and disease control of aquatic species (Defoirdt et al. 2011; Yaakob et al. 2014). This includes antioxidant activity (Sheikhzadeh et al. 2012), immunostimulatory (Newaj-Fyzul and Austin 2015), anti-inflammatory (Grammes et al. 2013), and antimicrobial effects (Austin and Day 1990; Falaise et al. 2016). Besides their nutritional and functional values, microalgae can be grown rapidly, often in saline or wastewater, and can make use of marginal land. Thus, cultivated microalgae could offer much higher areal productivities and offer products with a smaller ecological footprint, compared with terrestrial plants (Draaisma et al. 2013).

Microalgae have already been used as protein sources for human and animal consumption. The large-scale commercial production of microalgae began in the early 1960s (Garcia et al. 2017). *Spirulina* and *Chlorella* that contain protein up to 70% of the dry weight are the major commercialized microalgae, and some of their products are sold as protein supplements with additional nutritional components such as vitamins and carotenoids for humans (Wells et al. 2017). Microalgae-derived biomass has also been used to feed domestic animals (Becker 2007), and more than 40 species of microalgae are used in aquaculture (Pulz and Gross 2004). The most commonly used aquaculture species include strains from the genera *Chlorella*, *Tetraselmis*, *Isochrysis*, *Pavlova*, *Phaeodactylum*, *Chaetoceros*, *Nannochloropsis*, *Skeletonema* and *Thalassiosira* (Priyadarshani and Rath 2012). Traditionally, live microalgae are fed to the larvae of fish, crustaceans, and mollusks as well as zooplankton, which are produced as feed for fish larvae (Pulz and Gross 2004). Apart from feeding microalgae directly to fish larvae and zooplankton, incorporation of microalgae biomass into aquafeeds has been intensively studied in recent years (Shah et al. 2017). Microalgae can be either incorporated as whole cell ingredients or as extracted and processed components such as concentrated oil supplements (Maisashvili et al. 2015). Defatted microalgae biomass is a by-product of biofuel or high value compound production (e.g. carotenoids and omega-3 fatty acids), and can also be used as a protein source in aquafeed that additionally contains minerals, carotenoids, and other bioactive compounds (Ju et al. 2012; Kiron et al. 2016; Sørensen et al. 2017).

The chemical and nutritional composition of microalgae varies depending on the species, strains, and culture conditions. The suitability of microalgae as ingredients in fish feeds may therefore differ accordingly (Skrede et al. 2011). For example, the protein digestibility was 35.3%, 79.9% and 18.8% for *Nannochloropsis oceania*, *Phaeodactylum tricornutum* and *Isochrysis galbana* (Skrede et al. 2011). The apparent protein digestibility of feed containing *Schizochytrium* sp. was estimated be 87-88% in Atlantic salmon, when the dried alga was included at 0-15% of the diet (Kousoulaki et al. 2015). In another feeding study of Atlantic salmon parr, the DHA-rich oil from the thraustochytrid *Schizochytrium* sp. was able to replace 100% of fish oil in the diet, without any detrimental effect on growth (Miller et al. 2007). Numerous feeding trials confirmed

that microalgae have the potential to partially or completely replace fishmeal and fish oil. These results were reviewed by Shah et al. (2018), Roy and Pal (2015) and Hemaiswarya et al. (2011). Thus, microalgae are considered a promising future alternative to provide quality feed ingredients in aquaculture. Since the nutritional value of microalgae varies widely among different species, extensive research is needed to further develop knowledge about the physiology and nutritional properties of microalgae strains (Wells et al. 2017).

1.4 Industrial microalgae cultivation

Microalgae are excellent sources of feedstocks for both food and non-food products, but the key to successful industrialization of microalgae cultivation rests on the production of biomass at a large scale. Phototrophic microalgae can be cultivated in either open raceway ponds or closed photobioreactors (Fig. E). In both systems, light intensity and temperature are critical factors affecting the performance of microalgae cultures (Mata et al. 2010). Open raceway ponds are shallow, ring-shaped channel systems that are relatively easy to operate. These are the most common cultivation systems and have been already used for production of nutraceutical and food products (Kumar et al. 2015; Gellenbeck 2012). However, raceway pond systems are often not efficient due to their generally low surface area to volume ratio, and offer only low photosynthetic efficiency (1.5%) together with low biomass productivity and cell density (0.1-0.5g/L) (Kumar et al. 2015). Harvesting and dewatering microalgae from these systems could require high energy costs, mainly due to the dilute nature of microalgae cultures in open ponds (Uduman et al. 2010). Moreover, open systems are prone to contamination and can be affected by the weather conditions (Pérez-López et al. 2017). Therefore, strains cultivated in open systems must be able to cope with fluctuation in abiotic factors including temperature and salinity, and also possible invasion by competitors and predators. As a result, relatively few microalgae species have been cultivated in these systems. Since monoalgal and axenic cultures are often required for nutraceutical and pharmaceutical applications, closed photobioreactor systems have been developed (Posten 2009). In closed photobioreactors, environmental parameters such as temperature and pH can be controlled, hence these systems offer the best method to achieve high productivity and high cell

density (2-6 g/L), and to culture more sensitive microalgae with minimal contamination (Norsker et al. 2011; Draaisma et al. 2013). To maximize light energy utilization and production performance, various closed photobioreactors have been designed with large surface area to volume ratios and a short optical paths, including tubular or flat-plate systems (Posten 2009). Flat plate photobioreactors are amongst the most efficient photobioreactors that can reach ultrahigh cell density (>10g/L) (Richmond 2013) and consume less energy than tubular systems (Jorquera et al. 2010). The main disadvantage of closed photobioreactors is generally higher capital costs for installation and infrastructure compared with open ponds. However, photobioreactor systems are not necessarily more expensive in operation and this technology is still under development.

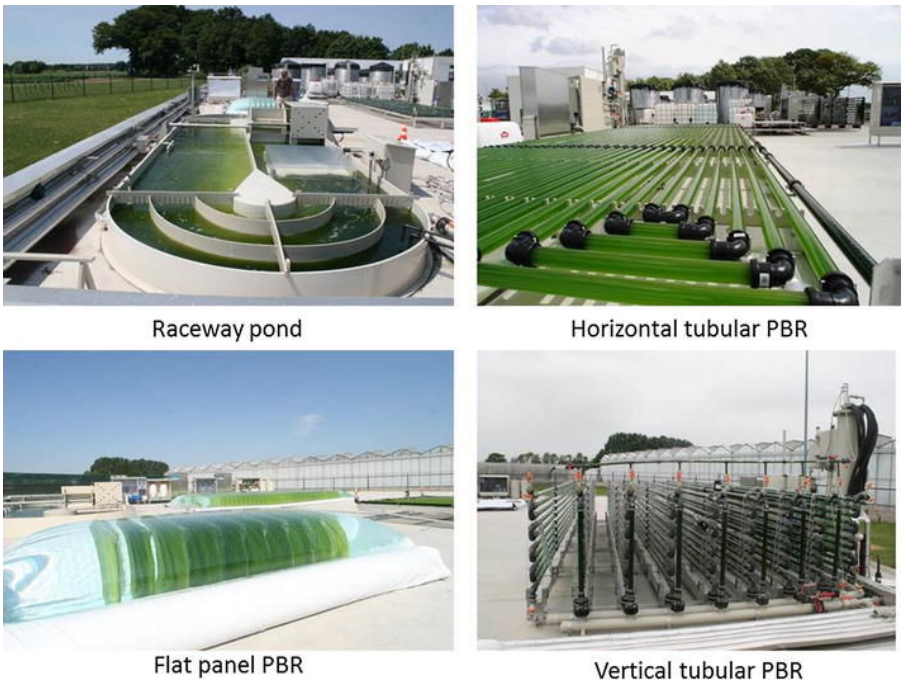

Fig E. Open raceway pond and closed photobioreactors (PBR) at AlgaePARC pilot facilities,Wageningen UR, the Netherlands (de Vree et al. 2015).

Although many studies have found that open ponds are more cost-efficient than closed systems (Davis et al. 2011; Richardson et al. 2012), some studies showed that the closed photobioreactors can be competitive with open raceway ponds systems for microalgae biomass production (Jorquera et al. 2010; Draaisma et al. 2013). Recent techno-economic analysis of microalgae for EPA and DHA production demonstrated that flat-plate photobioreactors have the lowest

production costs when compared with tubular and open ponds systems, with costs of 39.1 to 73.9 USD per kg total EPA and DHA in Spain and the Netherlands, respectively (Chauton et al. 2015). In the same study, the production cost increased by around 40% from flat-plate to tubular systems, and by around 80% from flat plate to open ponds systems. It has been estimated that the production cost could be reduced to 11.9 USD per kg total EPA and DHA by further optimization of biological productivity and engineering parameters in the next 5-10 years (Chauton et al. 2015).

2. Challenges of microalgae production in cooler climates

Despite the potential applications, successful large-scale industrial algae production is still rare. In cooler climates, commonly used temperate and warm-water microalgae display substantially lower yields or cannot survive (Pankratz et al. 2017; Moody et al. 2014, Fig. F). In nature, however, it has been estimated that approximately 72,500 species of algae exist (Guiry 2012). Among them, some microalgae have successfully colonized extremely cold environments such as glaciers, sea ice, polar and alpine regions (Varshney et al. 2015), and hence may exhibit higher biomass productivities. Bioprospecting and optimization of strains that are able to grow at low temperatures and contain high value compounds such as omega-3 fatty acids and pigments is critically required to develop microalgae cultivation in cooler climates (Kvíderová et al. 2017), and such work is only beginning.

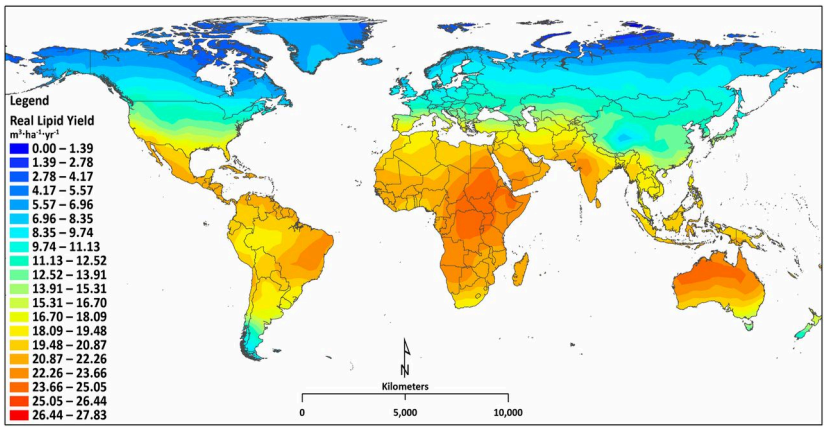

Fig F. World map of lipid productivity potential from microalgae based on biological growth model representative of *Nannochloropsis* cultivated in photobioreactors. The optimal temperature for growth of the strain: 26°C (Moody et al. 2014).

In previous work, five different cold-adapted snow and soil microalgae were tested, and the results showed that these strains could produce relatively high biomass and lipid yields at low temperatures of 6°C (Hulatt et al. 2017). Amongst the strains, *Raphidonema sempervirens* exhibited one of the highest productivities and also contained the long-chain omega-3 polyunsaturated acid, EPA. In this study, a closely related cold-adapted species *Koliella antarctica* (Trebouxiophyceae, Andreoli et al. 1998) was chosen to further investigate the potential biotechnological applications. Although the literature indicates that *K. anarctica* has promising features for biotechnological applications, including the production of LC-PUFAs (Lang et al. 2011), the best cultivation conditions for *K. antarctica* to obtain optimal growth and lipid production have not been reported. In particular, accurate information on its fatty acid profile, LC-PUFA content and TAG composition is not available. Therefore, we examined and optimized the growth and fatty acid production of *K. antarctica* in controlled experimental photobioreactors.

3. Objectives

The overall aim of this study was to investigate whether the cold-adapted microalga *K. antarctica* could be suitable for LC-PUFA production in cooler water temperatures. This thesis represents a contribution to the state of the art on the production of high value compounds, especially omega-3 and long chain fatty acids, from cold-adapted microalgae in photobioreactors. To achieve this goal, the study was divided into the following objectives:

1. Optimization of cultivation conditions for growth, including salinity and light intensity.
2. Characterization of the effect of nitrogen starvation, phosphorus starvation, and high salinity on protein content and the dynamics of fatty acids in TAG and polar lipids.
3. Evaluation of nutritional values for biotechnological applications.
4. Comparison of the maximum biomass productivity and photosynthetic efficiency of *K. antarctica* at cooler temperatures, with temperate strains.

These objectives were examined in the following manuscript.

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Manuscript

Growth and LC-PUFA production of the cold-adapted microalga *Koliella antarctica* in photobioreactors

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Abstract

Microalgae are excellent sources of polyunsaturated fatty acids (PUFAs), but only a few species have been thoroughly investigated in controlled photobioreactor conditions. In this work, the cold-adapted microalga *Koliella antarctica* (Trebouxiophyceae) was cultivated at 15 °C to optimize growth and PUFA production in bubble-tube and flat-plate photobioreactors. The impact of nitrogen starvation, phosphorus starvation, salinity and light intensity on the growth, fatty acid composition and protein content was investigated. After culture optimization, a maximum biomass productivity of 2.37 g L^{-1} d^{-1}, and maximum cell density of 11.68 g L^{-1} were achieved. Amongst all conditions tested, the maximum total fatty acid (TFA) content measured 271.9 mg g^{-1} dry weight in the late stationary phase. Nitrogen and phosphorus starvation strongly induced neutral lipid (TAG) accumulation, up to 90.3% of TFA, that mostly consisted of the monounsaturated fatty acid C18:1n-9 (oleic acid, OA). PUFAs were also abundant and together accounted for 30-45% of total TAG. The highest eicosapentaenoic acid (EPA, C20:5n-3) content amounted to 6.7 mg g^{-1} dry weight (4.9% TFA) in control treatments, whilst the highest arachidonic acid (ARA, C20:4n-6) content was 9.6 mg g^{-1} dry weight (3.5% TFA) in the late stationary phase. Phosphorus starvation was the best strategy to obtain high total fatty acid yields (mg L^{-1}) whilst maintaining the protein, total PUFA and long-chain omega-3 fatty acid contents.

Keywords: omega-3 fatty acid; LC-PUFA; eicosapentaenoic acid EPA; flat-plate photobioreactor; triacylglycerol; cold-adapted microalgae

1. Introduction

Microalgae are excellent sources of bioactive compounds that are known to benefit animal and human health (Borowitzka 2013). Among them are polyunsaturated fatty acids (PUFAs), particularly long-chain PUFAs (LC-PUFAs) with carbon chain lengths of C20 and above. Especially, eicosapentaenoic acid (EPA, C20:5n-3), docosahexaenoic acid (DHA, C22:6n-3) and arachidonic acid (ARA, C20:4n-6) are important dietary components for animals and humans (Khozin-Goldberg et al. 2011; Martins et al. 2013b). At present, LC-PUFAs are mostly derived from fish oils, but cultivated microalgae could provide "natural" and "healthy" vegetarian alternatives that have lower environmental impacts (Wijffels and Barbosa 2010; Chisti 2013; Khozin-Goldberg et al. 2011). Although research on microalgae-derived PUFAs is very promising, only a few species have been thoroughly investigated, and many of these are from temperate or warm-water habitats. Bioprospecting and optimization of strains that are able to grow rapidly at lower temperatures could also enable year-round utilization of culture facilities in temperate zones, and promote microalgae cultivation in cooler climates (Kvíderová et al. 2017).

Cold-adapted microalgae from polar habitats recently gained much attention not only for PUFA production (Spijkerman et al. 2012; Teoh et al. 2004), but also for their potential to achieve high productivity at lower water temperatures (Hulatt et al. 2017). Their evolutionary adaptations have enabled them to successfully colonize many low-temperature areas, including glaciers, sea ice, polar and alpine regions, which are often characterized by large seasonal fluctuations in environmental factors such as light and osmotic stress (Procházková et al. 2018; Morgan-Kiss et al. 2006). Such adaptations could benefit industrial cultivation systems, where microalgae often encounter fluctuations in light intensity, low temperature and variable salinity, each of which can impact culture productivity. Besides, the elevation of PUFA levels in membrane lipids is one of the key adaptive strategies that microalgae use to maintain cell metabolism at lower water temperatures (Morgan-Kiss et al. 2006; Boelen et al. 2013). Cold-adapted microalgae may therefore be more productive sources of PUFAs and other algae-derivatives that could be used as food or feed products.

In microalgae cell cultures, abiotic factors such as temperature, salinity, light and nutrient availability can be altered to induce high-value fatty acid production. Although physiological responses to these stressors are often species or strain-specific, the accumulation of fatty acids is often coincident with reduced growth rate, leading to an overall decrease in fatty acid productivity (Chen et al. 2017). Moreover, the main storage lipids in microalgae are triacylglycerols (TAG) that accumulate under stress conditions, and are often comprised by saturated and mono-unsaturated fatty acids (Sharma et al. 2012). This in turn alters microalgae oil quality and its nutritional profile, e.g. a decrease in the proportion of PUFAs. Hence, the best culture conditions should be carefully selected to maximize the production of PUFAs (Markou and Nerantzis 2013).

The cold-adapted microalga *Koliella antarctica* (Trebouxiophyceae, Andreoli *et al.*, 1998) is a potential candidate green alga for PUFA production due to the presence of LC-PUFAs (Lang et al. 2011). *Koliella antarctica* can grow at low temperatures down to 2 ˚C, and achieves maximum growth at 15 ˚C (Vona et al. 2004). However, the best cultivation conditions for *K. antarctica* to obtain optimal growth and lipid production are not reported. In particular, accurate information on its fatty acid profile, LC-PUFA content and TAG composition are not available.

In this work, we optimized the growth and fatty acid production of *K. antarctica* in controlled photobioreactors. The impact of nitrogen starvation, phosphorus starvation, salinity and light intensity on the growth, fatty acid composition and protein content of *K. antarctica* was investigated. Polar lipids and TAG were separated and changes in the fatty acid profiles of these fractions were characterized. The effect of light intensity on the growth and photosynthetic efficiency of *K. antarctica* was subsequently measured using flat-plate photobioreactors. Finally, the productivity of *K. antarctica* at 15 ˚C, adopting the optimal conditions, was compared with that of other temperate microalgae strains.

2. Materials and Methods

2.1 Cultivation

Koliella antarctica SAG 2030 (Chlorophyta, Trebouxiophyceae) was obtained from the Culture Collection of Algae at Göttingen University (SAG, Germany). Prior to experiments, the cultures were maintained in 250 mL Erlenmeyer flasks with Bolds Basal Medium (BBM, Bischoff and Bold, 1963). The cultures were illuminated with a light intensity of 90 ± 20 μmol m^{-2} s^{-1}, supplied by cool-white fluorescent lamps. A temperature of 15 ˚C was maintained throughout experiments, which is representative of summer conditions in cool climates, and winter temperatures in warmer parts of the world.

2.2 Experimental design

2.2.1 Salinity experiment in 100 mL bubble tubes

Koliella antarctica was cultivated in 27 mm diameter glass bubble tubes containing 80 mL of BBM (Table 1, Fig. 1) to which different amounts of artificial sea salt were added. Salinities 0, 2, 4, 8, 16, and 32 ‰ were tested, and cultures were illuminated at 80 μmol photons m^{-2} s^{-1} using white fluorescent lamps. Each treatment was maintained in a climate-controlled incubator (Termaks AS, Bergen, Norway) and the tubes with different treatments were randomized. Cultures were sparged with air enriched with 1.0% CO_2 at a flow rate of 100 mL min^{-1}, supplied by a precision gas mixer (Photon Systems Instruments, Drasov, Czech Republic) and delivered to each tube by individual air rotameters (FL-2000, Omega, Manchester, UK). Samples for biomass measurement were taken daily over a two-week cultivation period, and each treatment was performed in duplicate.

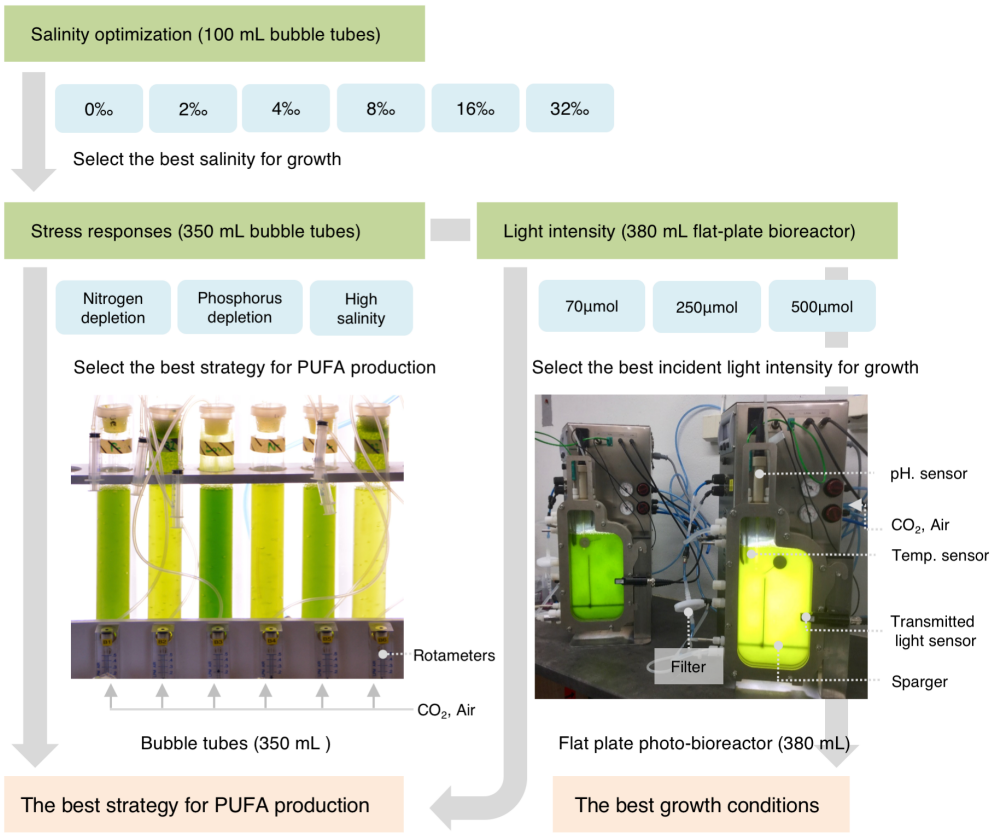

Fig 1. Experimental design and two types of photobioreactor used in the present study including bubble tubes (left) and flat-plate photobioreactors (right) with 14 mm light path length.

Table 1. Summary of experimental treatments in this study.

Experiment	Reactor type	Volume (mL)	Light intensity (µmol m^{-2} s^{-1})	Treatment	Salinity (‰)	Nutrient medium	CO_2 (%)
Salinity	Bubble tubes	100	80	-	0,2,4,8,16,32	BBM	1
Stress	Bubble tubes	350	120	Control	4	3N-BBM	1
Stress	Bubble tubes	350	120	N starvation	4	3N-BBM without nitrate	1
Stress	Bubble tubes	350	120	P starvation	4	3N-BBM without phosphate	1
Stress	Bubble tubes	350	120	HS	32	3N-BBM	1
Li-Ex I	Flat plate	380	70, 250, 500	-	4	2N-BBM	1
Li-Ex II	Flat plate	380	500	-	4	3× 3N-BBM	5

[a]BBM is Bold's Basal Medium in its original formulation, whilst 3N-BBM is the common adjustment where the nitrate concentration is increased 3-fold (3N). Additional adjustments were made for individual experiments, where 2N-BBM corresponds to double the concentration of nitrate, and 3× 3N-BBM is 3N-BBM formulation at three-fold its original concentration.
[b]The soil extract component of Bold's Basal Medium remained the same in all of the media in this study.

2.2.2 Stress experiment in 350 mL bubble tubes

In this experiment, larger 350 mL glass bubble tubes (Fig. 1) were used to test the effect of nitrogen (N-), phosphorus (P-) and high salinity (HS) stress conditions on growth, fatty acid and protein production. Cultures used in all experimental treatments were initially grown in the same 320 mL of 3N-BBM medium (BBM with 3-fold nitrate, see Table 1) supplied with 1.0% CO_2 at 120 µmol photons m^{-2} s^{-1}. When the cultures reached the exponential phase, the cells in each tube were centrifuged (3500 rcf, 5 min), washed and resuspended in one of four experimental media. The four treatments were (i) control (3N-BBM), (ii) nitrate free, N- (3N-BBM without $NaNO_3$), (ii) phosphate free, P- (3N-BBM without KH_2PO_4 or K_2HPO_4) and (iv) high salt, HS (3N-BBM with artificial sea salt added to 32 g L^{-1}) (Table 1). The time of washing and the application of experimental treatments was designated as the start of the experiment (t = 0). Each treatment was performed in triplicate and randomly assigned to one of the bioreactors.

2.2.3 Light experiments in flat-plate photobioreactors

Koliella antarctica was cultivated in 380 mL flat-plate photobioreactors (*Algaemist-S*, Ontwikkelwerkplaats, Wageningen UR, The Netherlands) shown in Figure 1. Prior to the experiments, the bioreactor and each medium (Table 1) were autoclaved (121 °C, 20 min). The reactor cultivation vessel with a 14 mm light path was illuminated continuously with warm-white LEDs from one side. Cultures were sparged with 0.2 µm filtered air (Acrodisc PTFE filters, Pall Corporation, New York, USA) enriched with either 1.0 or 5.0 % CO_2 (Table 1). The cultivation temperature was accurately controlled with an external cooling system (Julabo F25, JULABO GmbH, Seelbach, Germany) and an internal heating system, maintaining 15 °C ± 0.4. The photosynthetic photon flux density (PPFD, µmol m^{-2} s^{-1} PAR) incident on the front of the cultivation vessel was measured with a Li-Cor189 2π quantum sensor. The output PPFD (the light transmitted through the reactor) was determined by calibrating the photobioreactor light sensor with the average PPFD, which was measured at 28 positions with the LiCor sensor. In the first experiment (Li-Ex I) the PPFD on the front surface was 23 µmol m^{-2} s^{-1} for the first six days, to

avoid immediate light stress, then increased to either 70, 250 or 500 µmol m^{-2} s^{-1}. In the second experiment (Li-Ex II) with higher nutrient concentrations, only 500 µmol m^{-2} s^{-1} was supplied.

2.3 Growth measurements

The optical density was determined by measuring the absorbance at 540 and 680 nm in a 1 cm cuvette, using a spectrophotometer (Hach-Lange DR3900, Hach Lange GmbH, Düsseldorf, Germany). The samples were diluted with medium (1 to 50-fold) to maintain the absorbance reading below 1.0 for linear response. The dry weight was determined by filtering 10 mL of culture broth through pre-weighed ~1.0 µm pore size 47 mm glass fiber filters (VWR, Oslo, Norway). Filters were then rinsed with isotonic ammonium formate (0.5 M for salinity and stress experiment, 0.06 M for flat-plate photobioreactor) to remove extracellular salt and dried at 95 ˚C for 48 h. Filters were subsequently re-weighted and the dry weight (g L^{-1}) was calculated. The dry weight was calibrated with the absorbance at 540 nm as in Eq (1) (R^2 = 0.99, n = 26).

$$DW = (0.295 \cdot A_{540}) + 0.088 \quad (1)$$

Where DW is the calculated dry weight (g L^{-1}) based on the spectrophotometer absorbance at 540 nm (A_{540}). The absorbance ratio (A_{680}/A_{540}) was also determined to indicate the ratio of pigments (mainly chlorophyll) to the biomass. Samples for further biochemical analysis were obtained by pelleting cells in 2.0 mL microcentrifuge tubes, washing with isotonic ammonium formate to remove salt, then stored at -40 ˚C.

2.4 Nutrients

Samples for nitrate and phosphate analysis were centrifuged (3500 rcf, 5 min) and the supernatant was stored at -40 ˚C. The extracellular nitrate and phosphate concentration were measured with standard colorimetric methods as described by Ringuet et al. (2011). Nitrate was reduced to nitrite by NADH:nitrate reductase (NECi Superior Enzymes, Lake Linden, USA) and measured at 540 nm (FLUOstar Optima, BMG Lab-tech, Ortenberg, Germany) using a 96-well microplate. Extracellular phosphate was determined using the ascorbic acid/molybdate method, and the

absorbance of the colored complex was measured at 880 nm with a spectrophotometer (Hach-Lange DR3900). Nitrate and phosphate uptake rates (mM L^{-1} d^{-1}) were calculated by dividing the change in nutrient concentrations by the difference in time.

2.5 Fatty acid analysis of polar lipids and TAG

Total lipids were extracted from approximately 7 mg of freeze-dried biomass using 4.0 mL of chloroform:methanol solvent (2:2.5 v/v) containing internal standard (Tripentadecanoin, C15:0 Triacylglycerol, Sigma-Aldrich, Oslo, Norway). A bead mill (Precellys 24, Bertin Technologies, Montigny le Bretonneux, France) and 0.1 mm glass beads was used for the extraction. TAG and polar lipids were separated by solid-phase extraction with Waters Sep-Pak columns (1 g silica cartridges, 6 mL, Waters, Dublin, Ireland). Neutral lipids (TAG) were first eluted with 10 mL of 7:1 (v/v) hexane:diethylether, then polar lipids were eluted with 10 mL of methanol:acetone:hexane (2:2:1 v/v/v). Solvents were then evaporated under N_2 gas and the fatty acids were derivatized to fatty acid methyl esters (FAMEs) by adding acidic methanol (3.0 mL 5% H_2SO_4 in methanol, 70 °C for 3 hours). FAMEs were collected into hexane and quantified using a Gas Chromatograph equipped with a Flame Ionisation Detector (SCION 436, Bruker, Livingston, UK) and an Agilent CP-Wax 52CB column (Agilent technologies, Santa Clara, USA). Supelco 37 component standards (Sigma-Aldrich, Bellefonte, USA) were used for identification and quantification of the FAMEs. Additional standards were obtained to identify more unusual C16 and C18 series fatty acids.

2.6 Elemental composition and protein content

The elemental composition of biomass including carbon, hydrogen and nitrogen (CHN) was determined by elemental analysis (Elemental Microanalysis, Okehampton, UK). The protein content (%) was calculated using a nitrogen-to-protein conversion factor of N × 4.78 (Lourenço et al. 2004), where N is the elemental nitrogen content (%).

2.7 Calculations

The volumetric productivity (mg L^{-1} d^{-1}) was calculated between two time points, as in Eq (2).

$$P_i = \frac{C_{x.i} - C_{x.i-1}}{t_i - t_{i-1}} \quad (2)$$

Where P is the productivity, $C_{x.i}$ and $C_{X.i-1}$ are the concentrations of the biomass (g L^{-1}) at two time points and $t_i - t_{i-1}$ is the time between measurements. For the salinity experiment, the maximum productivity was calculated between days 5 and 7 ($t_i - t_{i-1} = 2$ days). In the stress experiment, the average productivity was calculated between days 0 and 5 ($t_i - t_{i-1} = 5$ days). In Li-Ex I and Li-Ex II, the maximum volumetric productivity during the cultivation was calculated as the highest productivity observed between two time points in each treatment. The maximum areal productivity P_A (g m^{-2} d^{-1}) was calculated according to Eq (3).

$$P_A = \frac{P_{max} \cdot V_R}{I_A} \quad (3)$$

where P_{max} is the maximum productivity (g L^{-1} d^{-1}), V_R is the reactor volume (0.38 L) and I_A is illuminated surface area (0.029 m^2). The maximum biomass yield per mol photons PAR ($Y_{x/mol}$, g mol^{-1}) was calculated using Eq (4).

$$Y_{X/mol} = \frac{P_A}{PPFD} \quad (4)$$

Where PPFD is the average PAR photon flux density on surface of the reactor (mol m^{-2} d^{-1}).

3.Results

3.1 Effect of salinity on growth in 100 mL bubble-tubes

The growth of *K. antarctica* in different salinities ranging from freshwater (0‰) to seawater (32‰) conditions is shown in Table 2 and Figure 1S. Higher growth was attained under low salinity conditions (<8‰). At salinity levels from 0 to 16‰, the maximum dry weight was in the range 2.69 to 2.90 g L^{-1}. However, at salinity 32‰ the maximum biomass concentration was lower, reaching only 2.14 g L^{-1}. The maximum productivity of 0.53 g L^{-1} d^{-1} was achieved by *K. antarctica* during exponential growth at salinity 4‰. Hence, 4‰ was selected as the optimal salinity for further cultivations.

Table 2. Maximum dry weight and biomass productivity of *K. antarctica* cultured under different salinities.

Salinity (‰)	Maximum dry weight (g L^{-1})	Maximum productivity (g L^{-1} day^{-1})
0	2.69 ± 0.03	0.51 ± 0.006
2	2.87 ± 0.10	0.50 ± 0.001
4	2.90 ± 0.02	0.53 ± 0.042
8	2.77 ± 0.03	0.46 ± 0.026
16	2.69 ± 0.06	0.48 ± 0.002
32	2.14 ± 0.14	0.45 ± 0.052

Values are mean ± standard error of duplicates.

3.2.1 Effect of different stressors on growth in 350 mL bubble-tubes

To select the best strategy that enables *K. antarctica* to produce high amounts of fatty acids and PUFAs, we investigated the impact of nitrogen (N-) starvation, phosphorus (P-) starvation and high salinity (HS) conditions on fatty acid and protein assimilation in two-stage cultures. After the cultures entered the exponential phase, the cells were washed and resuspended in different media for either N starvation, P starvation or HS conditions in the second stage (Table 1). Control cultures were resuspended in complete 3N-BBM nutrient medium. The growth curves of *K. antarctica* cultivated under the four conditions are shown in Fig 2a. In all conditions the biomass

concentration increased, but the growth pattern differed among the treatments. Control cultures displayed strong growth and achieved the highest dry weight of 3.7 g L^{-1}, at day 5. The lag phase in the HS treatment extended to 2 days, but the growth rate was eventually similar to that in the P-starved condition; both achieved similar maximum dry weights of 2.2 g L^{-1} and 2.3 g L^{-1}, respectively. The growth of *K. antarctica* under N starvation was much lower compared with the other treatments, where the maximum biomass reached only a quarter of that achieved in the control medium.

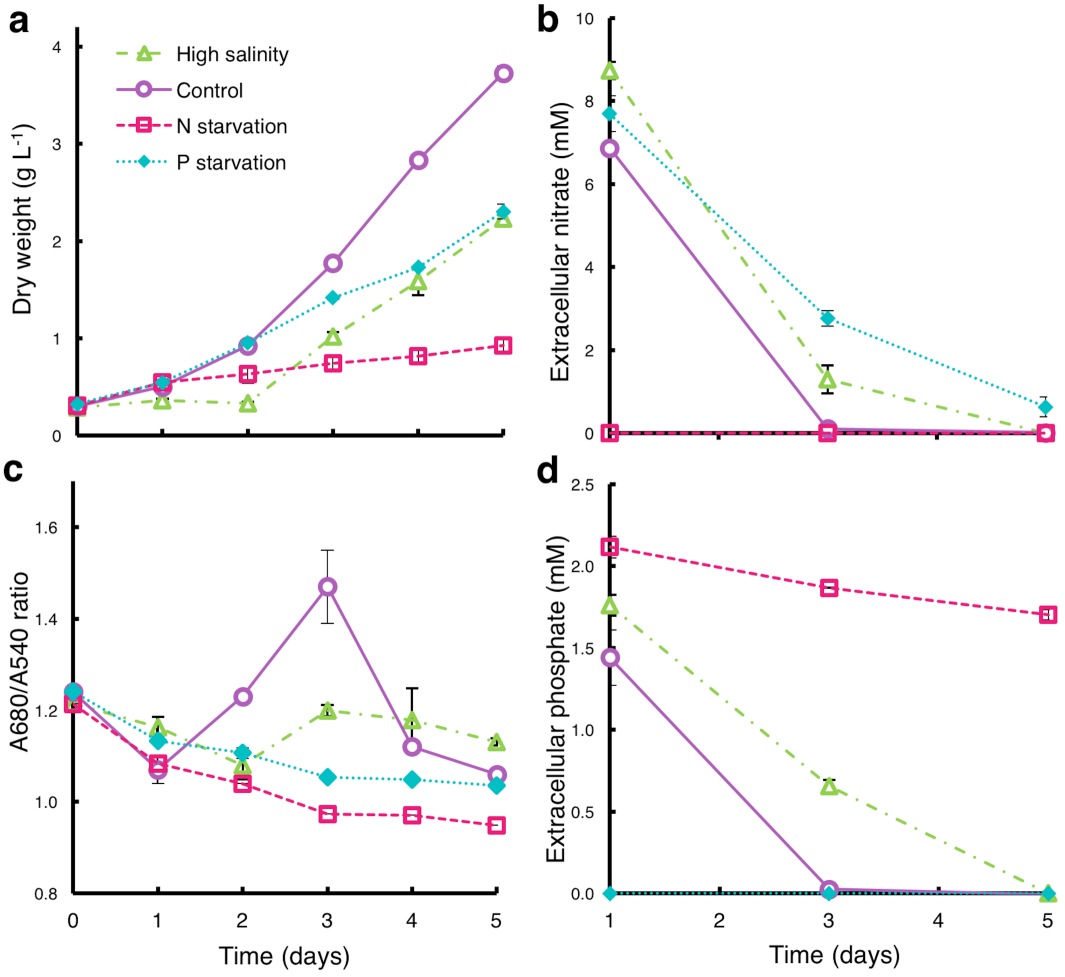

Fig 2. Effect of N starvation, P starvation and high salinity (32‰) on the growth and nutrient uptake of *K. antarctica* in batch cultures. (a) Cell density (g L^{-1}). (b) Extracellular nitrate concentration (mM). (c) Absorbance ratio (A_{680}/A_{540}). (d) Extracellular phosphate concentration (mM). The error bars indicate standard error of the mean (n = 3).

Figures 2b and 2d illustrate the nitrate and phosphate assimilation capacity of *K. antarctica* under different stress conditions, whilst the corresponding nutrient uptake rates are presented in Table 3. Phosphate uptake in the N-starved cultures was 0.10 mM d^{-1}, which was lower compared to those in the control (0.71 mM d^{-1}) and HS groups (0.55 mM d^{-1}). The nitrate uptake rate in P-starved cultures was 1.77 mM d^{-1}, which was about half of that in control group (3.38 mM d^{-1}). From day 0 to 5, the A_{680}/A_{540} ratio in both N and P starved media decreased, but N-starved cells were characterized by lower ratios compared with P-starved cells, indicating loss of photosynthetic pigments (Fig. 2c). At the end of cultivation, the values for N-starved cells and P-starved cells were 0.95 and 1.04, respectively (Fig. 2c). Due to rapid growth in the control treatments, nitrate and phosphate were each exhausted by day 3 (Fig. 2b, d). Similarly, the cells under HS conditions also consumed nitrate by day 5. Hence, *K. antarctica* in the control medium and 32‰ salinity condition still experienced N+P and N starvation respectively, during the later stages of cultivation.

3.2.2 Effect of different stressors on fatty acid and protein production in 350 mL bubble-tubes

Table 3 shows that N-starved and P-starved treatments produced the highest concentrations of total fatty acids (TFA) at day 5 (each 18 % of DW), but the reduced biomass productivity under N starvation ultimately led to the lowest fatty acid yield of 165.4 mg L^{-1}. In contrast, control cultures achieved high biomass productivity of 0.69 g L^{-1} d^{-1} and total fatty acids were 13.6% DW, leading to the highest TFA yield of 507.6 mg L^{-1}. Under P starvation, the protein content of *K. antarctica* was 31.4 ± 0.2 % DW, which was much higher than that under N starvation (12.2 ± 0.4 % DW) and control treatments (21.9 ± 0.7 % DW). Cells in N-starved conditions had a mean C:N ratio of 19.8 ± 0.4, approximately twice that of the other treatments, which ranged from 7.9 to 10.7.

Table 3. Effect of N starvation, P starvation and high salinity (32‰) on growth, biochemical composition, and nutrient assimilation. The average biomass productivities P_v (g L^{-1} d^{-1}) from day 0 to 5 and fatty acid content per volume of culture Y_{FA} (mg L^{-1}), nitrate uptake (mM d^{-1}) and phosphate uptake (mM d^{-1}) were calculated as described in the materials and methods. Fatty acid content C_{FA} (% DW), protein content C_{pro} (%DW) and the C:N ratio were measured at day 5.

Treatment	P_V (g L^{-1} d^{-1}) (day 0 to 5)	Y_{FA} (mg L^{-1}) (day 5)	C_{FA} (% DW) (day 5)	C_{pro} (% DW) (day 5)	C:N ratio (day 5)	Nitrate uptake (mM d^{-1})	Phosphate uptake (mM d^{-1})
Control	0.69 ± 0.01	507.6 ± 38.4	13.6 ± 0.8	21.9 ± 0.7	10.72 ± 0.34	3.38 ± 0.08	0.71 ± 0.08
N-	0.12 ± 0.00	165.4 ± 2.5	17.9 ± 0.2	12.2 ± 0.2	19.80 ± 0.37	-	0.10 ± 0.01
P-	0.40 ± 0.01	418.4 ± 32.4	17.8 ± 0.7	31.4 ± 0.1	7.94 ± 0.04	1.77 ± 0.06	-
HS	0.39 ± 0.02	241.5 ± 12.6	10.8 ± 0.5	27.0 ± 0.7	7.95 ± 0.26	4.35 ± 0.07	0.55 ± 0.03

Mean values and standard errors of triplicate measurements are given.

In all treatments the total fatty acid content increased from day 2 to 5, mainly due to TAG accumulation (Table 4). The highest proportion of TAG was detected under N starvation at day 5 and accounted for 79.5% of the TFA (179.1 mg g^{-1} DW). In nutrient-replete conditions (control day 2), the dominant fatty acids were C16:0, C16:4n-3, C18:3n-3 (α-linolenic acid, ALA) and C20:5n-3 (EPA). Under N and P starved conditions, C16:0, C18:1n-9 (oleic acid, OA), C18:2n-6 (linoleic acid, LA) and ALA became dominant. TAG accumulation was attributed predominantly to a large increase in OA, comprising up to 52.7% of TAG. However, the PUFAs, LA, ALA and C20:4n-6 (arachidonic acid, ARA) were also enriched in TAG, and together total PUFAs accounted for 33.8-45.9% of TAG at day 5. The highest PUFA content of 90.6 mg g^{-1} DW, as well as the highest ω-3 fatty acid content of 43.6 mg g^{-1} DW, was observed after 5 days of P starvation. The LA and ALA contents were also higher in P-starved cells compared with other treatments. The highest amount of EPA was 6.7 mg g^{-1} DW (4.9% TFA) in high-density control cultures (day 5), but lower during P and N starvation at 5.4 and 3.8 mg g^{-1} DW, respectively. The lowest EPA content of 2.1 mg g^{-1} DW was recorded for the HS group at day 2. In contrast to EPA, the ARA content was enhanced more by N starvation than by P starvation. ARA increased from 1.4 mg g^{-1} DW in nutrient replete conditions (4.0% TFA) to 7.4 and 4.6 mg g^{-1} DW (4.1 and 2.6% TFA) in N and P treatments, respectively, due to its accumulation in TAG. The HS treatment induced the accumulation of TAG with a large proportion of OA at day 2. The proportion of TAG was higher in the HS treatment (35.0% TFA) than that of control cultures (20.2% TFA).

Table 4. Total fatty acids (TFA) and fatty acids in TAG under N starvation, P starvation and HS (32‰) conditions (mg g^{-1} DW).

	Control				**N-**				**P-**				**HS**			
	day 2		**day5**		**day 2**		**day5**		**day 2**		**day5**		**day 2**		**day5**	
	TFA	**TAG**	**TFA**	**TAG**	**TFA**	**TAG**	**TFA**	**TAG**	**TFA**	**TAG**	**TFA**	**TAG**	**TFA**	**TAG**	**TFA**	**TAG**
C14:0	0.54	0.04	1.17	0.38	0.46	0.28	1.13	0.55	0.41	0.11	1.26	0.58	0.31	0.02	1.14	0.26
	(0.05)	(0.01)	(0.03)	(0.04)	(0.17)	(0.03)	(0.06)	(0.01)	(0.09)	(0.03)	(0.06)	(0.03)	(0.08)	(0.02)	(0.06)	(0.01)
C16:0	3.62	0.36	19.16	10.71	7.42	5.31	17.23	11.47	6.66	2.20	19.24	7.21	3.19	1.39	16.51	7.45
	(0.28)	(0.03)	(1.30)	(1.17)	(1.04)	(0.74)	(0.50)	(0.27)	(0.96)	(0.19)	(1.90)	(0.22)	(0.81)	(0.21)	(1.02)	(0.19)
C16:2(n-6)	0.64	0.05	6.56	2.04	2.57	0.79	4.43	1.99	1.57	0.37	6.42	2.71	0.61	0.12	5.48	1.22
	(0.06)	(0.02)	(0.04)	(0.12)	(0.26)	(0.11)	(0.22)	(0.06)	(0.17)	(0.05)	(0.33)	(0.08)	(0.08)	(0.01)	(0.34)	(0.06)
C16:3(n-3)	1.69	0.16	5.48	2.11	5.04	1.72	6.97	3.87	3.82	0.94	7.79	4.11	1.63	0.23	5.31	1.44
	(0.09)	(0.02)	(0.07)	(0.13)	(0.46)	(0.22)	(0.30)	(0.13)	(0.50)	(0.13)	(0.40)	(0.09)	(0.24)	(0.03)	(0.29)	(0.02)
C16:4(n-3)	6.16	1.35	5.14	2.42	3.36	1.22	3.87	2.14	4.59	1.41	4.89	2.76	3.79	0.72	5.03	1.73
	(0.32)	(0.08)	(0.11)	(0.12)	(0.33)	(0.17)	(0.17)	(0.09)	(0.59)	(0.19)	(0.19)	(0.09)	(0.66)	(0.09)	(0.35)	(0.11)
C18:0	0.32	0.29	1.61	0.48	0.00	0.23	1.61	0.60	-0.04	0.31	2.47	0.62	0.00	0.02	2.11	0.34
	(0.14)	(0.15)	(0.54)	(0.22)	(0.14)	(0.06)	(0.44)	(0.08)	(0.06)	(0.02)	(0.64)	(0.11)	(0.17)	(0.04)	(0.34)	(0.04)
C18:1(n-9)	0.53	0.13	37.03	32.85	25.20	23.74	78.78	75.05	7.71	6.34	57.56	52.69	2.43	1.86	22.55	19.30
	(0.03)	(0.01)	(3.82)	(3.96)	(3.37)	(3.24)	(2.07)	(2.45)	(0.71)	(0.56)	(2.03)	(1.96)	(0.34)	(0.26)	(0.49)	(0.34)
C18:1(n-7)	0.24	0.00	1.09	0.65	0.51	0.35	1.31	1.01	0.50	0.26	2.18	1.44	0.13	0.02	0.58	0.29
	(0.01)	(0.00)	(0.05)	(0.05)	(0.06)	(0.05)	(0.03)	(0.03)	(0.05)	(0.03)	(0.13)	(0.07)	(0.04)	(0.02)	(0.05)	(0.02)
C18:2(n-6) (LA)	1.55	0.17	20.45	11.34	8.92	5.71	20.63	15.44	6.57	2.97	27.05	16.75	2.02	0.94	15.74	7.28
	(0.06)	(0.01)	(0.74)	(1.04)	(1.08)	(0.79)	(0.32)	(0.55)	(0.71)	(0.32)	(1.05)	(0.51)	(0.29)	(0.13)	(0.77)	(0.17)
C18:3(n-6)	0.47	0.13	2.93	1.78	2.13	1.50	4.29	3.76	2.56	0.98	8.90	4.45	0.52	0.26	2.27	1.22
	(0.04)	(0.01)	(0.09)	(0.13)	(0.27)	(0.20)	(0.07)	(0.12)	(0.32)	(0.13)	(0.49)	(0.11)	(0.08)	(0.03)	(0.08)	(0.03)
C18:3(n-3) (ALA)	11.46	1.50	16.80	7.49	12.94	5.61	19.85	11.63	12.98	4.45	22.66	13.46	8.43	1.87	16.94	5.88
	(0.64)	(0.11)	(0.36)	(0.54)	(1.28)	(0.66)	(0.72)	(0.48)	(1.66)	(0.57)	(0.93)	(0.40)	(1.33)	(0.24)	(1.03)	(0.22)

C18:4(n-3)	2.52	0.76	1.79	0.93	0.75	0.40	0.98	0.63	2.14	0.85	2.85	1.92	1.35	0.37	1.87	0.73
	(0.15)	(0.05)	(0.04)	(0.05)	(0.08)	(0.06)	(0.04)	(0.02)	(0.31)	(0.13)	(0.11)	(0.04)	(0.24)	(0.05)	(0.12)	(0.03)
C20:1(n-9)	0.46	0.00	5.09	3.09	2.65	1.81	6.84	5.52	1.18	0.82	4.90	3.91	0.77	0.28	3.80	1.94
	(0.02)	(0.00)	(0.38)	(0.38)	(0.34)	(0.24)	(0.10)	(0.20)	(0.10)	(0.05)	(0.17)	(0.13)	(0.13)	(0.03)	(0.12)	(0.02)
C20:4(n-6) (ARA)	1.42	0.55	4.79	2.54	3.19	2.20	7.37	5.74	1.72	1.12	4.63	3.29	1.06	0.58	3.61	1.97
	(0.09)	(0.03)	(0.17)	(0.18)	(0.41)	(0.33)	(0.05)	(0.18)	(0.22)	(0.16)	(0.17)	(0.10)	(0.17)	(0.05)	(0.15)	(0.07)
C20:5(n-3) (EPA)	3.74	1.70	6.71	4.37	2.83	2.07	3.78	3.03	3.01	2.07	5.44	4.08	2.06	1.18	5.42	3.48
	(0.25)	(0.12)	(0.17)	(0.21)	(0.35)	(0.28)	(0.01)	(0.08)	(0.37)	(0.27)	(0.15)	(0.08)	(0.35)	(0.17)	(0.20)	(0.07)
Σ SFA	4.48	0.68	21.94	11.57	7.73	5.82	19.98	12.62	7.03	2.62	22.98	8.41	3.34	1.43	19.75	8.06
	(0.22)	(0.19)	(1.87)	(1.39)	(1.29)	(0.82)	(0.86)	(0.26)	(1.09)	(0.20)	(2.51)	(0.13)	(1.05)	(0.26)	(1.42)	(0.24)
Σ MUFA	1.23	0.13	51.68	36.59	28.36	25.90	86.92	81.58	9.39	7.42	64.64	58.03	3.33	2.16	26.93	21.53
	(0.03)	(0.01)	(4.23)	(4.39)	(3.77)	(3.54)	(2.14)	(2.68)	(0.86)	(0.63)	(2.27)	(2.15)	(0.50)	(0.30)	(0.61)	(0.33)
Σ PUFA	29.65	6.38	73.75	35.02	41.73	21.21	72.17	48.24	38.96	15.18	90.64	53.52	21.47	6.26	61.68	24.95
	(1.68)	(0.45)	(1.56)	(2.48)	(4.50)	(2.79)	(1.63)	(1.62)	(4.82)	(1.94)	(3.80)	(1.48)	(3.43)	(0.78)	(3.11)	(0.57)
Σ LC-PUFA	5.16	2.24	12.03	6.92	6.01	4.28	11.15	8.77	4.73	3.20	10.07	7.36	3.13	1.76	9.03	5.45
	(0.34)	(0.15)	(0.28)	(0.38)	(0.76)	(0.59)	(0.06)	(0.25)	(0.59)	(0.44)	(0.32)	(0.18)	(0.52)	(0.23)	(0.25)	(0.03)
ω3 fatty acids	25.57	4.40	35.92	9.25	24.92	6.89	35.46	10.45	26.55	5.49	43.64	11.58	17.26	3.93	34.58	7.51
	(1.45)	(0.17)	(0.66)	(5.01)	(2.49)	(2.64)	(1.21)	(6.20)	(3.42)	(1.25)	(1.78)	(7.34)	(2.82)	(0.32)	(1.95)	(3.27)
ω 6 fatty acids	4.08	0.90	34.72	17.69	16.81	10.20	36.71	26.94	12.42	5.45	47.00	27.19	4.21	1.89	27.09	11.69
	(0.23)	(0.07)	(0.97)	(1.47)	(2.01)	(1.41)	(0.46)	(0.86)	(1.40)	(0.65)	(2.02)	(0.79)	(0.62)	(0.22)	(1.16)	(0.14)
ω3/ω6 Ratio*	6.27	6.10	1.04	0.98	1.49	1.09	0.97	0.79	2.13	1.78	0.93	0.97	4.08	2.30	1.27	1.13
	(0.01)	(0.07)	(0.02)	(0.03)	(0.03)	(0.02)	(0.02)	(0.01)	(0.04)	(0.02)	(0.00)	(0.00)	(0.11)	(0.05)	(0.02)	(0.02)
Total fatty acids	**35.36**	**7.20**	**135.80**	**83.18**	**77.82**	**52.94**	**179.08**	**142.44**	**55.38**	**25.22**	**178.25**	**119.96**	**28.14**	**9.86**	**108.36**	**54.54**
	(1.84)	(0.27)	(7.65)	(8.22)	(9.53)	(7.15)	(2.10)	(4.51)	(6.76)	(2.76)	(7.29)	(3.73)	(4.96)	(1.32)	(4.97)	(0.83)

Mean values (±standard error) of triplicates per treatment are given in the table.

3.3.1 Effect of light intensity on growth in 380 mL flat-plate photobioreactors

The effect of light intensity on the growth of *K. antarctica* was studied in flat-plate photobioreactors. In the first experiment (Li-Ex I), the maximum biomass productivity increased from 0.48 to 1.04 g L^{-1} d^{-1} with rising irradiance from 70 to 250 μmol m^{-2} s^{-1} (Table 5, Fig. 3), but there was no further increase in growth rate when light was increased to 500 μmol m^{-2} s^{-1}. Extracellular nitrate in all the cultures was exhausted by day 8 (Fig. 3c). In cultures supplied with 250 and 500 μmol m^{-2} s^{-1}, the A_{680}/A_{540} ratio decreased rapidly after day six, reaching 0.95 to 0.93 after nine days. In cultures supplied with 70 μmol m^{-2} s^{-1} though, the effect was dampened, and the A_{680}/A_{540} ratio reached a lowest value of 0.98 at day 16 (Fig. 3b). The pH under 250 and 500 μmol m^{-2} s^{-1} increased from pH 6.5 ± 0.1 to pH 7.8 ± 0.1 in the first seven days, then decreased slightly to pH 7.6 toward the end of cultivation (Fig. 3d). In the 70 μmol m^{-2} s^{-1} treatments, the pH peaked at 7.8 ± 0.2 at day 8, and steadied at 7.7 ± 0.1 during the rest of the cultivation period. In Li-Ex I the attenuated growth under 500 μmol m^{-2} s^{-1} (Table 5), together with the nitrate and pH data, indicated that nutrient and CO_2 availability might have limited maximum growth in higher light conditions. To test whether we could obtain higher yields, the nutrient and CO_2 concentrations were subsequently increased, and the experiment was repeated at 500 μmol m^{-2} s^{-1}. In this second experiment (Li-Ex II), the maximum biomass productivity was substantially higher, reaching maximum 2.37 g L^{-1} d^{-1} (Fig. 4, Table 5). After the first day of cultivation, the absorbance ratio A_{680}/A_{540} in Li-Ex II remained between 0.95 and 1.09.

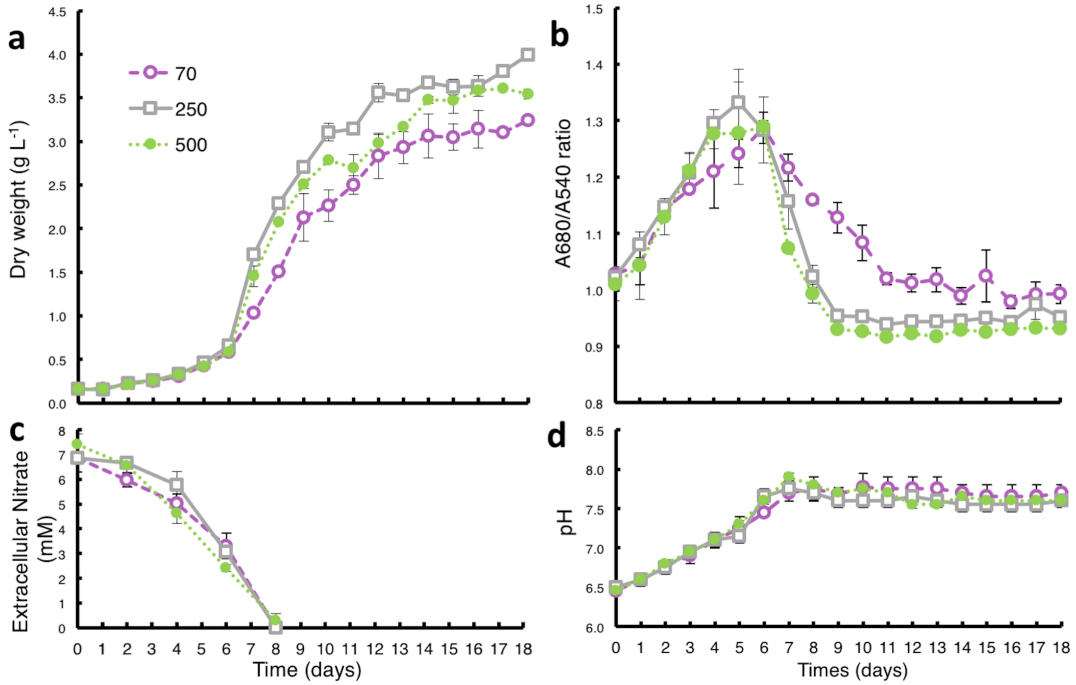

Fig 3. The effect of light intensity (70, 250 and 500 µmol m^{-2} s^{-1}) on the growth of *K. antarctica* in flat-plate photobioreactors over 18 days. (a) Cell density (g L^{-1}). (b) Extracellular nitrate concentration (mM). (c) The absorbance ratio A_{680}/A_{540}. (d) The culture pH. The error bars indicate the standard error of duplicate cultivations.

Table 5. Biomass production of *K. antarctica* grown in flat-plate photobioreactors illuminated with 70, 250 or 500 µmol m^{-2} s^{-1}. Values are the maximum biomass productivity (g L^{-1} d^{-1}), maximum areal productivity (g m^{-2} d^{-1}), and maximum photosynthetic yield (g mol^{-1}) obtained from the experiments Li-Ex I and Li-ExII.

Medium	Light intensity (µmol photons m^{-2} s^{-1})	CO_2 (%)	Maximum productivity (g L^{-1} day^{-1})	Maximum areal productivity (g m^{-2} day^{-1})	Maximum biomss yield on light (g mol^{-1} photons)
2N-BBM	70	1	0.48 ± 0.01	6.36 ± 0.08	1.052 ± 0.014
2N-BBM	250	1	1.04 ± 0.01	13.85 ± 0.07	0.641 ± 0.003
2N-BBM	500	1	0.87 ± 0.10	11.62 ± 1.39	0.269 ± 0.032
3× 3N-BBM	500	5	2.37 ± 0.09	31.58 ± 1.21	0.731 ± 0.028

Mean values (±standard error) of duplicates per treatment are given in the table.

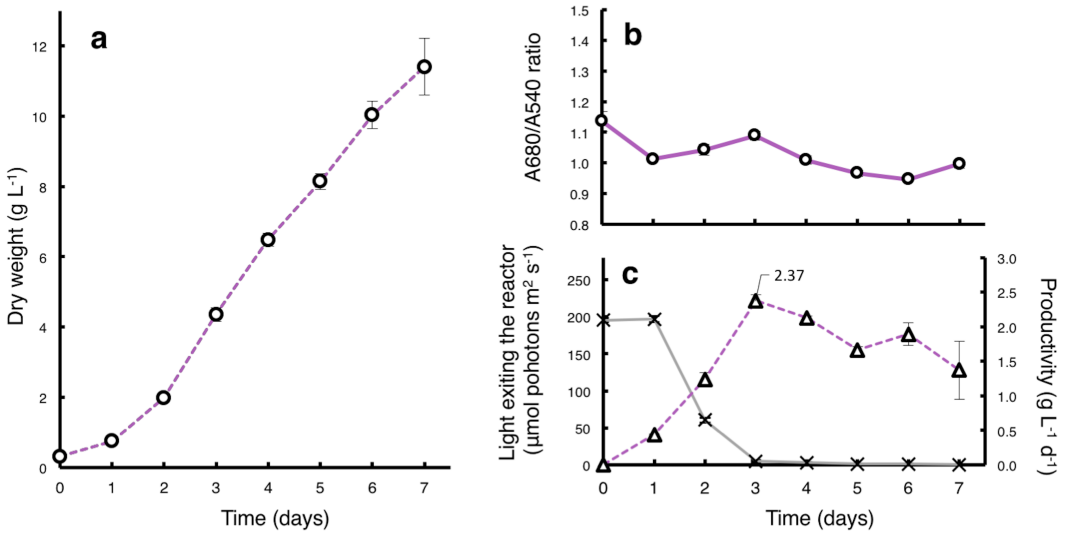

Fig 4. Growth of *K. antarctica* in optimized conditions in flat-plate photobioreactors (Li-Ex II, 500 µmol m^{-2} s^{-1}), using increased amounts of nutrients (3×3N-BBM) and CO_2 concentration (5% CO_2 v/v). (a) Cell density (g L^{-1}). (b) Absorbance ratio, A_{680}/A_{540}. (c) light transmitted through the reactor vessel (µmol m^{-2} s^{-1}) and biomass productivity (g L^{-1} d^{-1}). Error bars indicate the standard error of the mean values (*n*=2).

3.3.2 Photosynthetic yield in 380 mL flat-plate photobioreactors

To characterize the photosynthetic efficiency of *K. antarctica* under different light intensities, the maximum biomass yield per mol photons PAR ($Y_{x/mol}$, g mol^{-1}) was calculated for each treatment (Table 5). The cultures under low irradiance used light more efficiently than those at higher light intensities, averaging 1.05 g mol^{-1} at 70 µmol m^{-2} s^{-1}, and reducing to 0.27 g mol^{-1} under highest light intensity of 500 µmol m^{-2} s^{-1}. In Li-Ex II, *K. antarctica* was found to tolerate the strong illumination and high nutrient concentrations (Fig. 4a). The highest cell density of 11.68 g L^{-1} was obtained at the end of cultivation. In this second experiment, the photosynthetic efficiency at 500 µmol m^{-2} s^{-1} was improved to 0.73 g mol^{-1} and the maximum areal productivity of 31.58 g m^{-2} d^{-1} was the highest recorded (Table 5).

3.3.3 Fatty acid production in 380 mL flat-plate photobioreactors

In the flat-plate photobioreactors, *K. antarctica* illuminated with 70, 250 and 500 µmol m^{-2} s^{-1} was able to accumulate considerable amounts of fatty acids, with a large increase in TAG and a slight decrease in polar lipid fatty acids towards the stationary phase (Fig. 5, Fig 2S). The highest TFA of 271.9 mg g^{-1} DW was recorded under 250 µmol m^{-2} s^{-1} at day 18, where 90.1% TFA was found in TAG (Fig. 2S). The abundance of PUFAs in polar lipids decreased as cultures aged, but those in TAG largely increased (Fig. 5c). Total PUFAs at day 10 (late exponential phase) ranged from 52.9 to 53.9 mg g^{-1} DW and consistently increased up to 88.5 mg g^{-1} DW until day 18 in the late stationary phase. The highest total PUFA content was recorded when cultures were illuminated with 250 µmol m^{-2} s^{-1}, where 86.2% of total PUFAs were found in TAG. In all light treatments, we observed a time-dependent increase in ARA and LA content of *K. antarctica*, mostly attributed to TAG accumulation (Fig. 5a, 5b). The proportion of LA partitioning into TAG increased from 50.2 to 87.2% of total LA, whilst the proportion of ARA partitioning into TAG increased from 58.5 to 81.2% of total ARA. The highest ARA content was obtained under 250 µmol m^{-2} s^{-1} at 9.6 mg g^{-1} DW (3.5% TFA) in the late stationary phase. The total EPA content was mostly conserved throughout growth, ranging between 2.9 and 4.1 mg g^{-1} DW (Fig. 5a). However, the percent share of EPA consistently decreased from 3.6% of TFA during the late exponential phase, to its lowest value of 1.5% of TFA in the late stationary phase. The ratio of ω-3 to ω-6 fatty acids was the highest at 1.0 ± 0.1 at day 10, but decreased thereafter to 0.5 ± 0.03, irrespective of the light intensity (Fig. 2S), due to a large increase in the abundance of LA (Fig. 5b).

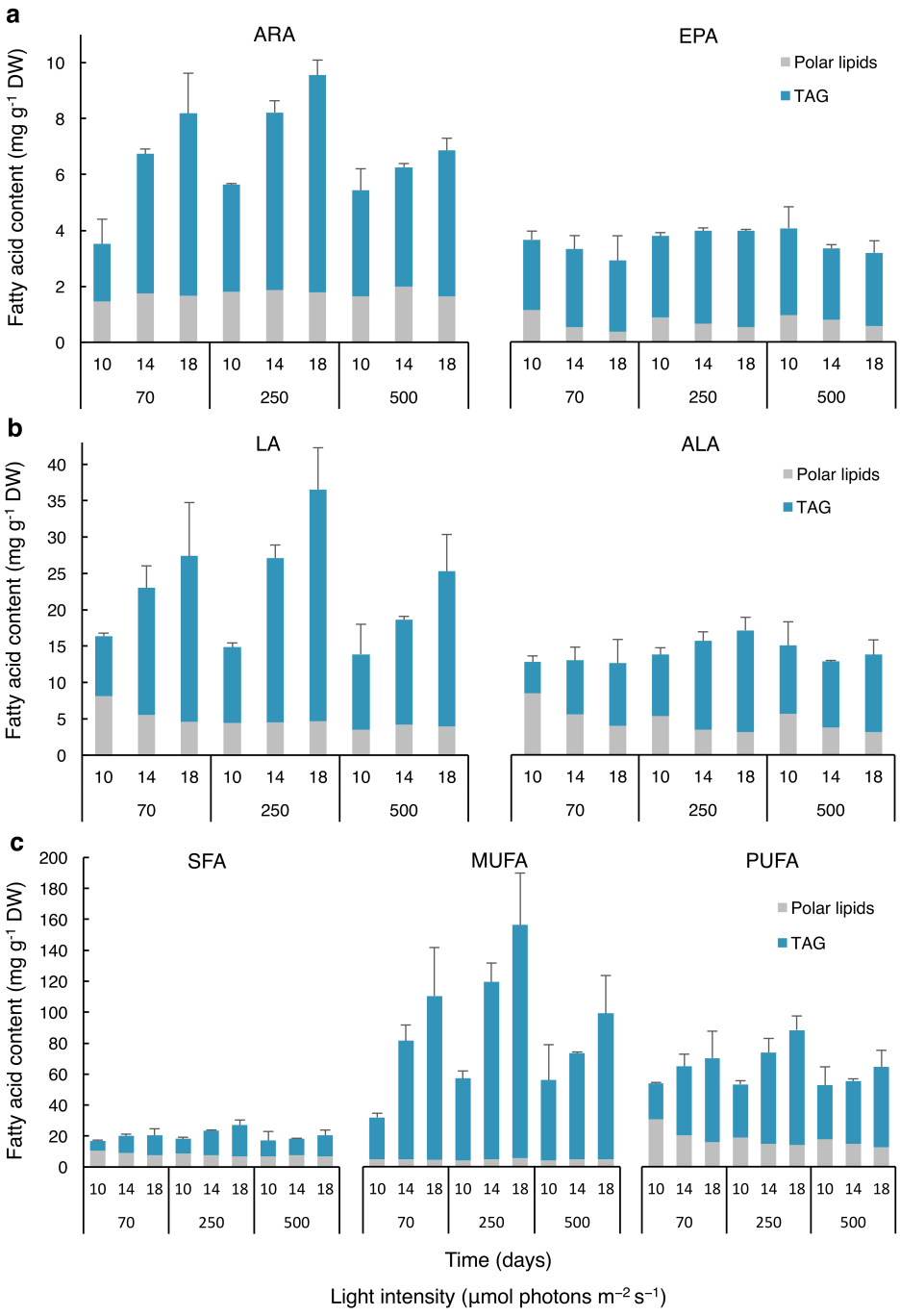

Fig 5. The fatty acid composition (mg g^{-1} DW) in TAG and polar lipids of *K. antarctica* grown in flat-plate photobioreactors under different light intensities (70, 250 and 500 µmol m^{-2} s^{-1}), determined at day 10, 14 and 18. (a) Arachidonic acid, C20:4n-6 (ARA, left) and Eicosapentaenoic acid, C20:5n-3 (EPA, right). (b) Linoleic acid (LA, left) and α-linolenic acid (ALA, right). (c) Saturated fatty acids (SFA, left), monounsaturated fatty acids (MUFA, center) and polyunsaturated fatty acids (PUFA, right). Error bars indicate the standard error of duplicate cultivations.

3.4 Comparison of growth of *K. antarctica* with other temperate strains

To determine whether the obtained maximum productivity of *K. antarctica* at 15 ˚C under optimal conditions was similar to that of other temperate strains, the results were compared with other studies that employed only flat-plate photobioreactors with short light paths (< 30 mm) and illuminated between 500 and 1000 µmol m^{-2} s^{-1} (Fig. 6). The productivity of *K. antarctica* at 15 ˚C was found to be comparable to that of the temperate strains cultivated at 20-35 ˚C, and the optimized maximum productivity (Li-Ex II) is amongst the higher values.

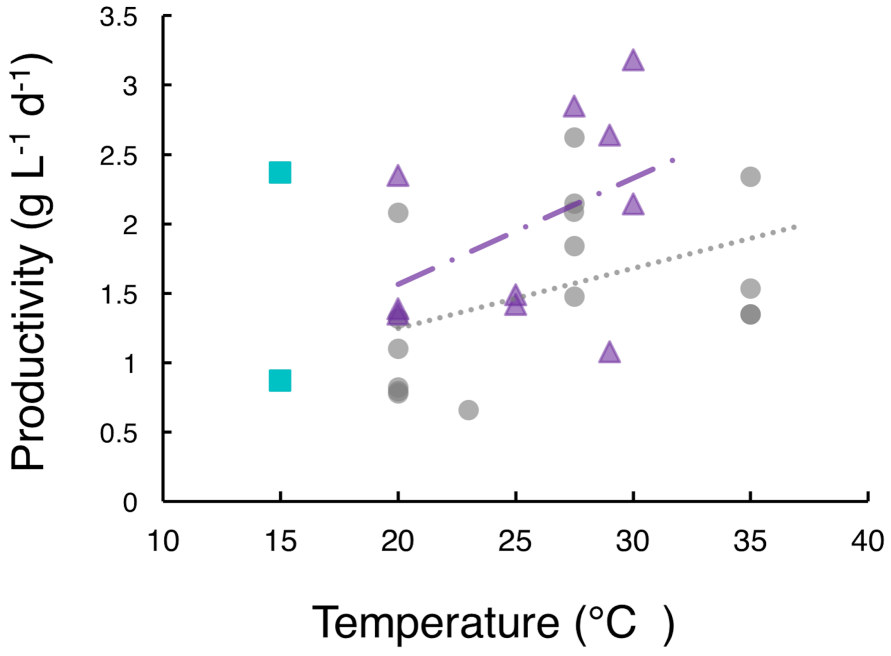

Fig 6. Comparison of maximum productivity of *K. antarctica* under 500 µmol m^{-2} s^{-1} at 15°C (■) with other studies (see Table 1S) under 500 µmol m^{-2} s^{-1} (●), or 500-1000 µmol m^{-2} s^{-1} (▲) in similar flat-plate bioreactors with comparable light path lengths (< 30mm). The grey and yellow lines represent the respective linear regression slopes.

4.Discussion

Although some data is available for *K. antarctica* (La Rocca et al. 2015; Vona et al. 2004; Ferroni et al. 2007; Lang et al. 2011), the present study provides a comprehensive analysis of its growth and fatty acid profile in photobioreactors. Especially, we were able to obtain relatively high biomass productivity and identify the effects of nutrient supply, high salt and light intensity on the dynamics of PUFA and TAG production. *K. antarctica* synthesized long-chain polyunsaturated fatty acids, in addition to the more common C16 and C18 series PUFAs, making this cold-water strain a potential candidate for producing food and feed ingredients.

4.1 *Koliella antarctica* is euryhaline, but prefers lower salinity conditions

Salinity can affect the growth of microalgae by altering the biochemical composition and photosynthetic function (von Alvensleben et al. 2016). To improve biomass productivity, the salinity should be optimized for each strain (Martínez-Roldán et al. 2014). Batch growth in bubble tubes indicated that *K. antarctica* preferred lower salinity conditions, with maximum growth rates at 4‰. However, the microalga maintained wide salinity tolerance up to natural seawater concentrations (0-32‰). Although *K. antarctica* was isolated from sea water in Terra Nova Bay, Ross Sea, Antarctica (Andreoli et al. 1998), based on morphological and physiological traits it was suggested that the microalga could have originated from Antarctic freshwater habitats (Andreoli et al. 2000; Ferroni et al. 2007). This microalga is not found in open seas, and our salinity tolerance data suggests the association of *K. antarctica* with terrestrial, freshwater or brackish habitats. This broad salinity tolerance offers flexibility in mass cultivation using saline or brackish water, and could help reduce the freshwater footprint linked to microalgae cultivation (Guieysse et al. 2013). Furthermore, the tolerance to salinity variations may also help to avoid significant decreases in outdoor productivity or culture collapse caused by evaporation or rainfall (Ishika et al. 2017).

4.2 Effect of nitrogen, phosphorus and high salinity stress on fatty acid production

Nitrogen starvation is often the most effective strategy to trigger fatty acid accumulation in microalgae, whilst phosphorus starvation typically has more limited effects (Chen et al. 2017). Salinity can also be manipulated to induce fatty acid accumulation (Pal et al. 2011; Salama el et al. 2013). However, these conditions often decrease or arrest growth, which results in lower overall fatty acid productivity (Procházková et al. 2014). We firstly compared the effect of N-, P- and HS on TFA yield (mg L^{-1}). Phosphorus-starved cultures showed the highest TFA yield of 418.4 mg L^{-1}, although the highest fatty acid concentrations were obtained in both N- and P-starved cells (each approximately 18% DW). However, the reduced growth in N-starved cultures led to the lowest TFA yield of only 165.4 mg L^{-1}. Although this result indicates that P starvation could be the most efficient strategy to obtain high fatty acid productivity in this strain, the profile of nutritionally valuable LC-PUFAs should also be a criterion for bioprocess optimization.

To further investigate the impact of different stressors on the fatty acid profile, total fatty acids were fractionated into neutral lipids (TAG) and polar (membrane) lipids. Although TAG accumulation is mostly studied for biofuel purposes (Hu et al. 2008), its importance as a valuable source of fatty acids in nutrition applications has also been investigated (Klok et al. 2014). *Koliella antarctica* accumulated TAG up to 90.0% of TFA. Although TAG was mostly comprised of the mono-unsaturated fatty acid OA, PUFAs were also abundant and accounted for around 30-45% of the TAG. This trend is similar to our previous study, where Arctic snow algae deposited comparable amounts of PUFAs in TAG (Hulatt et al. 2017). Incorporation of PUFAs into TAG, including LC-PUFAs, has also been reported in *Thalassiosira pseudonana* and *Pavlova lutheri* (Guiheneuf and Stengel 2013; Tonon et al. 2002). *Lobosphera incisa,* a green microalga isolated from an alpine environment, is also known to accumulate large amounts of ARA in its TAG. For some microalgae that inhabit harsh environments, TAG might serve as a depot of PUFAs (Bigogno et al. 2002), and these strains could be cultivated under adverse conditions to maximize PUFA production.

For animal and human health, foods with high ω-3 fatty acid content are desirable (Abedi and Sahari 2014). In addition, a balanced ω-3/ω-6 ratio is also important, because ω-3

and ω-6 fatty acids and their derivatives are often functionally and metabolically antagonistic (Glencross 2009; Simopoulos 2016). The recommended ω-3/ω-6 ratio for human health is 0.5 to 1.0 (Simopoulos 2016), and in our study the ω-3/ω-6 ratio of *K. antarctica* was between 0.5 and 6.3, under all conditions.

The best quality fatty acid profile was produced by P starvation amongst all the conditions tested. Phosphorus-starved cells contained the highest amount of PUFAs (90.6 mg g^{-1} DW, 50.9% TFA) and ω-3 fatty acids (43.6 mg g^{-1} DW, 24.5% TFA), with an ω-3/ω-6 ratio of 0.9. The essential fatty acids LA and ALA were also abundant, compared with the other stress treatments. Although OA constituted a third (32.3%) of the TFAs in P-starved cells, valuable PUFAs can be separated from other fatty acids by fractional distillation or winterization (Mendes et al. 2007; Cuellar-Bermudez et al. 2015). Both natural and refined microalgae oils could be used as sources of food and feed and in nutraceutical and pharmacological applications (Adarme-Vega et al. 2012).

4.3 *Koliella antarctica* produces the LC-PUFAs EPA and ARA

Only a subset of microalgae species have the necessary metabolic pathways to produce LC-PUFAs with chain lengths of C20 and beyond (Muhlroth et al. 2013). Therefore, strains such as *K. antarctica* that can produce EPA and ARA, are potentially valuable cell factories. Our fatty acid identifications are concordant with Lang et al. (2011), who also identified the production of ARA and EPA in *Koliella*. In this study the highest EPA content amounted to 6.7 mg g^{-1} DW (4.9% TFA) in control treatments. However, this was almost matched by cells under P starvation and HS conditions, each of which contained comparable amounts of EPA at 5.4 mg g^{-1} DW (3.1 and 5.0% of TFA, respectively). The proportion of EPA produced by *K. antarctica* was similar to *Tetraselmis chuii*, a microalga commonly used in aquafeeds, in which EPA accounted for 5.0% of TFA (Lang et al. 2011). ARA accumulation by *K. Antarctica* was induced by both N and P starvation, increasing up to 7.4 and 4.6 mg g^{-1} DW (4.1 and 2.6% TFA), respectively. The highest ARA content of 9.6 mg g^{-1} DW (3.5% of TFA) was found under 250 μmol m^{-2} s^{-1} after prolonged

nutrient starvation in the flat-plate photobioreactor, which is comparable to other candidate strains for ARA production, including *Porphyridium cruentum* (Řezanka et al. 2014).

EPA can be synthesized via the ω-3 and ω-6 pathways in microalgae (Guschina and Harwood 2006). Here, the presence of the intermediate fatty acid ARA implicates at least a role for the ω-6 route in *K. antarctica*. This pathway is also the dominant metabolic pathway in the EPA-rich eustigmatophyte *Nannochloropsis* (Schneider and Roessler 1994; Shene et al. 2016). However, the exact nature of LC-PUFA biosynthesis in *K. antarctica*, including the regulation of metabolic pathways, merits further investigation.

4.4 Protein content under nutrient stress

Nutritional and toxicological tests have reported that microalgae biomass is often suitable as a feed supplement or could replace conventional protein sources (Yaakob et al. 2014). Under stress conditions, carbon fixed by microalgae is partitioned toward carbohydrate or lipid synthesis, rather than protein synthesis (Hu et al. 2008). Our results indicate that phosphorus-starved cells had the highest protein content of 31.4% DW, whilst the nitrogen-starved cells recorded the lowest protein content of 12.2%. *K. antarctica* can accumulate fatty acids under P starvation whilst simultaneously maintaining the protein content. This feature could make the alga a suitable whole-cell ingredient for animal and fish feeds, in which both high protein and lipid contents are desired (Wells et al. 2017). However, the nutritional value of protein in *K. antarctica* needs to be evaluated by assessing its amino acid profile and conducting digestibility studies on animals.

4.5 *Koliella antarctica* is productive under high irradiance in flat-plate photobioreactors

Light intensity influences cell growth, and changes the biochemical composition of microalgae (He et al. 2015). Here, the maximum biomass yield on light was highest (1.05 g mol^{-1}) at 70 µmol m^{-2} s^{-1}, but decreased to 0.27 g mol^{-1} at 500 µmol m^{-2} s^{-1}. At low irradiances, higher photosynthetic efficiency can be obtained because heat dissipation by non-photochemical quenching (NPQ) or chlorophyll fluorescence are minimized (Müller et al. 2001). The maximum biomass yield on

light at 70 μmol m^{-2} s^{-1} was comparable to those obtained in other studies at warmer temperatures, 25 ˚C. For example, values of 1.25 g mol^{-1} and 1.11 g mol^{-1} were obtained at low light intensities in the case of *Chlamydomonas reinhardtii* (Takache et al. 2010; Kliphuis et al. 2012). The attenuated growth at 500 μmol m^{-2} s^{-1} relative to 250 μmol m^{-2} s^{-1} could be attributed to the combined effects of nutrient/CO_2 supply coupled with high irradiance. The high rate of nitrogen consumption in Li-Ex I likely suppressed protein biosynthesis and impaired photosynthetic performance (Ho et al. 2012). The negative effects of high irradiance can be mitigated by supplying excess nutrients and CO_2, and increasing the cell density to improve mutual shading (Chen et al. 2011). In Li-Ex II the maximum productivity of *K. antarctica* was substantially improved to 2.37 g L^{-1} d^{-1} and the maximum biomass yield on light was enhanced proportionally to 0.73 g mol^{-1}. The tolerance of the microalga to high light in Li-Ex II indicates that *K. antarctica* could be suitable for outdoor cultivation where cells may experience moderate to strong irradiance.

Productivity and yield data are often specific to the test conditions, or to the optical configuration of the apparatus used for cultivation. To account for these variables, the productivity of *K. antarctica* at 15 ˚C was compared with other studies using flat-plate photobioreactors that were illuminated between 500 and 1000 μmol m^{-2} s^{-1}, mostly at warmer temperatures. The results showed that the maximum productivity of 2.37 g L^{-1} d^{-1} at 15 ˚C was comparable to that of other strains at 20-35 ˚C. To our knowledge, this is amongst the higher biomass productivities obtained at temperatures ≤15 ˚C, and one of few studies that has used optimized photobioreactor platforms to cultivate polar microalgae.

4.6 Conclusions

The cold-adapted microalga *K. antarctica* exhibited high maximum biomass productivity of 2.37 g L^{-1} d^{-1} at 15 ˚C after culture optimization, and tolerated a relatively broad range of salinities. The highest total fatty acid content obtained in this work was 271.9 mg g^{-1} dry weight. Nitrogen and phosphorus starvation strongly induced TAG accumulation up to 90.3% TFA, that mostly consisted of the monounsaturated fatty acid OA. However, PUFAs were also abundant and together accounted for around 30-45% of total TAG. The highest amount of EPA was 6.7 mg g^{-1}

DW (4.9% TFA) in the control treatments. ARA accumulation mostly occurred in TAG and was largely induced by nitrogen starvation, reaching 9.6 mg g^{-1} DW (3.5% TFA) in the late stationary phase. Phosphorus starvation was the best strategy tested here to obtain high total fatty acid yields (mg L^{-1}) whilst maintaining the protein content. Phosphorus-starved cells also contained the highest total PUFAs and long-chain omega-3 fatty acids. The high productivity of *K. antarctica* at cooler temperatures concurrent with production of high value LC-PUFAs could make this strain a potential candidate for producing food and feed ingredients, possibly offering the opportunity for cultivation in cooler climates or during winter in temperate regions.

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Author contributions

HS planned and conducted the study with guidance from CJH, KV and RHW. HS analyzed the samples and data with assistance from CJH. HS wrote the manuscript with input from CJH, KV and RHW.

Competing Interests

The authors declare no competing interests

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Supplementary data

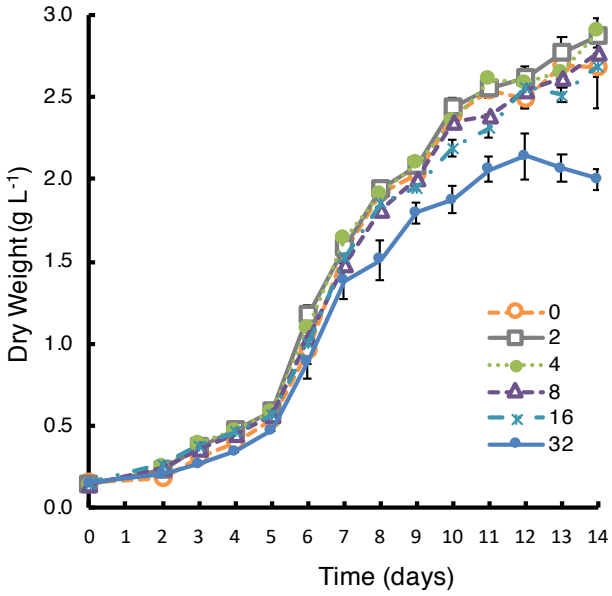

Fig 1S. The growth curves of *K. antarctica*, cultured for 14 days, under various salinity concentrations (0-32‰). Standard errors of the mean values of duplicates are shown.

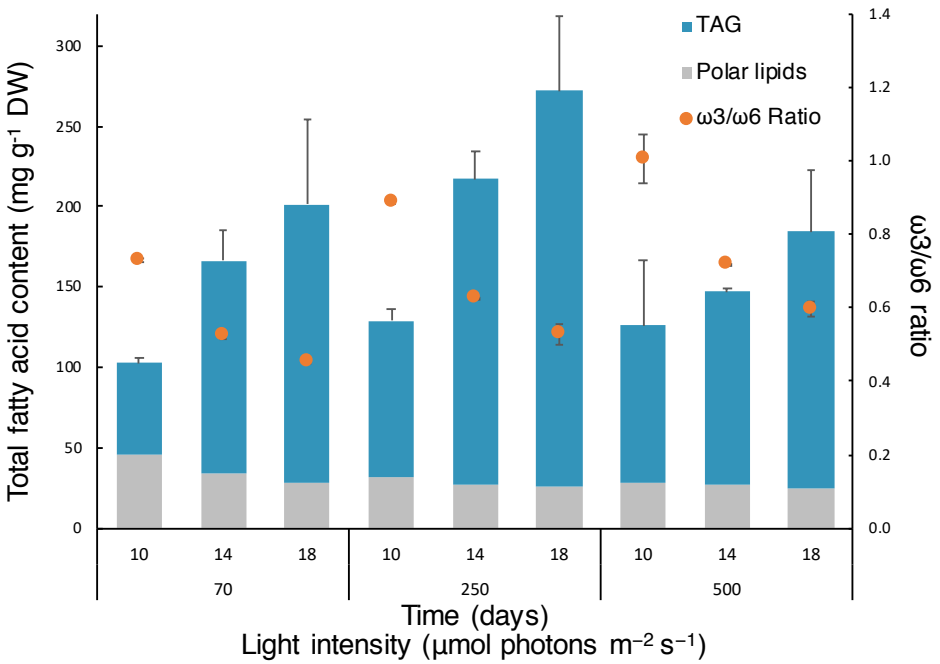

Fig 2S. Total fatty acid content (mg g^{-1} DW) in TAG and polar lipids and theω3/ω6 fatty acids ratio of *K. antarctica* grown in flat-plate photobioreactors under different light intensities (70, 250 and 500 µmol m^{-2} s^{-1}), determined at day 10, 14 and 18. Error bars indicate the standard error of duplicate cultivations.

Table 1S. Comparison of maximum biomass productivity of *K. antarctica* with literature values. The data are presented in manuscript Figure 6.

Species	Maximum biomass productivity ($g\ L^{-1}\ d^{-1}$)	Temperature (°C)	Light intensity ($\mu mol\ m^{-2}\ s^{-1}$)	Reference
Koliella antarctica	0.87	15.0	500	This study
Koliella antarctica	2.37	15.0	500	This study
Scenedesmus obliquus	0.8	20.0	500	Breuer et al. (2013)
Scenedesmus obliquus	0.8	20.0	500	Breuer et al. (2013)
Scenedesmus obliquus	0.8	20.0	500	Breuer et al. (2013)
Scenedesmus obliquus	2.1	20.0	500	Breuer et al. (2013)
Scenedesmus obliquus	1.3	20.0	500	Breuer et al. (2013)
Phaeodactylum tricornutum	1.1	20.0	500	Meiser and Walter Trösch. (2004)
Phaeodactylum tricornutum	1.4	20.0	750	Meiser and Walter Trösch. (2004)
Phaeodactylum tricornutum	1.4	20.0	1000	Meiser and Walter Trösch. (2004)
Chlorella vulgaris	1.4	25.0	560	Liao et al. (2017)
Scenedesmus obliquus	1.8	27.5	500	Breuer et al. (2013)
Scenedesmus obliquus	2.6	27.5	500	Breuer et al. (2013)
Scenedesmus obliquus	2.1	27.5	500	Breuer et al. (2013)
Scenedesmus obliquus	1.5	27.5	500	Breuer et al. (2013)
Scenedesmus obliquus	2.8	27.5	800	Breuer et al. (2013)
Chlorella vulgaris	1.1	29.0	980	Degen et al. (2001)
Chlorella vulgaris	2.6	29.0	980	Degen et al. (2001)
Scenedesmus ovalternus	3.2	30.0	750	Koller et al. (2018)
Scenedesmus ovalternus	2.1	30.0	750	Koller et al. (2018)
Scenedesmus obliquus	1.3	35.0	500	Breuer et al. (2013)
Scenedesmus obliquus	2.3	35.0	500	Breuer et al. (2013)
Scenedesmus obliquus	1.3	35.0	500	Breuer et al. (2013)
Scenedesmus obliquus	1.5	35.0	500	Breuer et al. (2013)

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