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Scaling-Up and Semi-Continuous Cultivation of Locally Isolated Marine Microalgae *Tetraselmis striata* in the Subtropical Island of Gran Canaria (Canary Islands, Spain)

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Abstract: The goal of this study was to determine the feasibility of the large-scale cultivation of locally isolated *Tetraselmis striata* in different open ponds in Gran Canaria. The biomass productivities were 24.66 ± 0.53 kg_{DW} in 32 days (28.9 t/ha/year) for 8000 L indoors, 42.32 ± 0.81 kg_{DW} in 43 days (38.8 t/ha/year) for an 8000 L pond outdoors, and 54.9 ± 0.58 kg_{DW} in 28 days (19.6 t/ha/year) for a 45,000 L pond outdoors. The photosynthetic efficiencies were 1.45 ± 0.03% for an 8000 L pond indoors, 1.95 ± 0.04% for 8000 L outdoors. and 1.10 ± 0.01% for a 45,000 L pond outdoors. The selected strain was fast-growing (μ = 0.21 day^{-1}) and could be rapidly scaled up to 45,000 L; it formed healthy cultures that maintained high photosynthetic activity during long-term cultivation and provided stable biomass productivities, able to grow on urea, which acted as a cheap and effective grazer control. The obtained biomass is a good source of proteins and has an FA profile with a high content of some nutritionally important fatty acids: oleic, α-linolenic (ALA) and EPA. The high ash content in the biomass (>35%) can be reduced by the implementation of additional washing steps after the centrifugation of the culture.

Keywords: marine microalga; outdoor raceways; photosynthetic efficiency; biomass production; long term cultivation; grazer control

Citation: Gojkovic, Z.; Guidi, F.; Bustamante, B.; Venuleo, M.; Assunçao, P.A.C.J.d.; Portillo, E. Scaling-Up and Semi-Continuous Cultivation of Locally Isolated Marine Microalgae *Tetraselmis striata* in the Subtropical Island of Gran Canaria (Canary Islands, Spain). *Processes* **2021**, *9*, 1326. https://doi.org/10.3390/pr9081326

Academic Editors: Francisco Gabriel Acién Fernández and Juan Luis Gomez Pinchetti

Received: 28 June 2021
Accepted: 26 July 2021
Published: 29 July 2021

1. Introduction

Mass outdoor cultures of marine microalgae are considered the most promising way to produce foods, feeds, bio-fertilizers, bio-stimulants and biofuels, among other products. They can be established on non-arable land, using poor-quality water, including seawater, brackish water, and waste water, as well as CO_2 from flue gas for their growth [1–4]. *Tetraselmis striata* belongs to *Chlorodendrophyceae*, a small class of green algae (Chlorophyta) with only two genera: *Tetraselmis* and *Scherffelia* [5]. *Tetraselmis* species are quadriflagellate, motile, solitary oval cells (10–25 µm long and 7–20 µm wide), containing a single cup-shaped chloroplast, a shield-shaped prominent nucleus located near the flagellar base and a large pyrenoid located in the chloroplast [5]. *Tetraselmis* sp. is widely used in aquaculture as feed for bivalve molluscs, shrimp larvae and rotifers because it is a highly adequate source of proteins, carbohydrates, lipids and fatty acids, essential for these organisms [6,7]. *Tetraselmis* species are also very interesting candidates for the production of natural pigments—violaxanthin, lutein and β-carotene are used for nutraceutical and cosmetics applications [8]. Moreover, biomass from microalga *Tetraselmis chuii* was recently approved as a novel food appropriate for human consumption [9]. *Tetraselmis* sp. can withstand ambient temperatures ranging from 5 to 40 °C and grow in wastewater and seawater with salinities of up to 75 g/L [8]. The high salinity tolerance of *Tetraselmis* sp. can be used to eliminate potential contaminants from large-scale cultures via salinity and

osmotic pressure shifts, given that contaminants are sensitive to osmotic stress [3]. All-year operation of most algal mass cultures is possible in the Mediterranean and similar climatic regions, with the exception of winter months due to the need to maintain the culture above 15 °C, as it is suggested that low temperatures and radiation limit the biomass productivities of *Tetraselmis* sp. [3]. There is no marked seasonality in the Canary Islands, which allows for the stable year-round production of most microalgal species, and gives an advantage in comparison with other climate regions with moderate climate zones, where seasonal variations of solar irradiance and temperature often lead to lower microalgal growth during winter compared to the summer season [10]. Nitrogen and phosphorus are the two main nutrients that play an essential role in and have a significant effect on the growth of *Tetraselmis* sp., and it is suggested that the highest biomass concentration and biomass productivity are obtained under high nitrogen concentrations (1.76 mM), with limited vitamin and low phosphorus (18 µM) concentrations [11]. *Tetraselmis* spp. readily utilizes various forms of inorganic nitrogen: nitrate, nitrite and ammonium, as well as organic nitrogen sources such as urea and organic acids. Once they have entered the cell, molecules containing N are reduced to ammonium and assimilated into amino acids through a variety of pathways [12]. Only in some specific applications are photoautotrophic algal cultures totally axenic, while in all mass cultivation systems, including open raceways and closed photobioreactors (PBRs), algae form a consortium with naturally occurring bacteria that can also enter the culture as airborne contaminants [13]. The interactions between the microalga and the associated bacterial community can promote cultural growth, and it was suggested that non-axenic *T. suecica* grow similarly with and without vitamins, thanks to the positive bacterial influence on the algal growth via nutrient exchange and recycling [13].

This study was formulated and conducted to obtain useful data on the large-scale production of *Tetraselmis striata*, to ultimately develop a process that can be used by microalgae companies in the Canary Islands. Our experimental hypothesis, based on preliminary evidence acquired in the ITC facilities, is that this native strain of *T. striata* is appropriate for viable large-scale cultivation in the Canary Islands, and represents a good candidate for commercial uses (e.g., production of aquaculture feed).

The main goal of this study was to determine the feasibility of the large-scale, long-term cultivation of the locally isolated marine microalga *T. striata* in various open ponds on Gran Canaria Island. For this purpose, we compared *T. striata* cultures over one-month (or longer) semi-continuous cultivations in medium-sized open ponds (8000 L) inside and outside a greenhouse, and afterwards we scaled up the *T. striata* culture to an outdoor 45,000 L open pond. Experiments were performed continuously from August to December 2019. This was the first study of large-scale, long-term *T. striata* cultivation in open ponds in the Canary Islands to date. Our results suggest that year-round large-scale biomass production using *T. striata* is possible, with stable biomass productivities (28.9 t/ha/year) and the absence of grazer contamination due to the addition of urea in the culture medium. One of the issues we came upon is the high ash content in the biomass obtained in outdoor ponds (>35%), which can be solved by the implementation of one additional washing step after the centrifugation of the culture.

2. Materials and Methods

2.1. Microalgae and Cultivation Conditions

Marine green microalga *T. striata* (GenBank access ID: MT012288, https://www.ncbi.nlm.nih.gov/nuccore/MT012288, accessed on: 25 July 2021) was sampled in Pozo Izquierdo (Gran Canaria, Spain) from desalination brine coming from the nearby desalination plant MSGC. It was isolated and identified at the Instituto Tecnológico de Canarias (ITC), Gran Canaria, Spain, and later deposited in the ITC collection with the code ITC-TETRA-03. *T. striata* was maintained in f culture medium [14], composed of natural (UV-treated and filtered) seawater, with the following composition (in mg/L): $NaNO_3$, 150; CON_2H_4, 60; $NaH_2PO_4 \cdot 2H_2O$, 11.30; Na_2EDTA, 4.16; $FeCl_3 \cdot 6H_2O$, 3.15; $CuSO_4 \cdot 5H_20$, 0.01; $ZnSO_4 \cdot 7H_20$, 0.022; $CoCl_2 \cdot 6H_20$, 0.01; $MnCl_2 \cdot 4H_20$, 0.18; $Na_2MoO_4 \cdot 2H_20$, 0.006. In addition to sodium

nitrate as the main nitrogen source, 1mM of urea was added to the medium (60 mg/L) to serve as both an additional N source and as a grazer-preventative [15]. The initial nitrogen concentration in the culture medium was 3.76 mM (1.76 mM in the form of nitrate + 2 mM in the form of urea). This concentration of N in medium is sufficient to support biomass concentrations of up to 0.67 g/L, based on the assumption of 7.9% N in the dry algal biomass [16,17]. The microalgae were aseptically cultured inside the indoor growth chamber at a controlled temperature (T = 25 ± 1 °C), and illuminated continuously with cool white LED light at a photon flux density of 300 $\mu mol_{photons}/m^2/s$, then bubbled with 1 L/L/min of air mixed with 1% CO_2. The bubbled chamber cultures (8 L) were subsequently used to inoculate 250 L raceways in a greenhouse, which were later used as the inoculum for 8000 L ponds (RW80in and RW76out), as described in detail in Section 2.3.

2.2. Measurements of the Optical Density, Quantum Productivity and Biomass Concentration of the Cultures

The optical density (OD) of the culture was used as an indicator of the daily growth performance. OD was determined at a wavelength of 750 nm using a UV/Visible spectrophotometer (HACH Lange DR3900) in a 10 mm light path polystyrene cuvette. Photosystem II's (PSII) maximum quantum productivity (Q_y) was determined by measuring the chlorophyll fluorescence in a portable pulse amplitude-modulation (PAM) fluorimeter (AquaPen AP-100, Photon Systems Instruments, Brno, Czech Republic) according to [18]. Q_y was measured daily and used as an indicator of culture photosynthetic performance and health. The biomass concentration was determined as described in [19] after filtration of a known culture volume over pre-dried and pre-weighted glass fiber filters (Whatman GC) by measuring the weight increase in the dried filters. The biomass on filters was washed twice with 150 mL of ammonium formate (NH_4HCO_2) isoosmolar to the culture medium to remove excess salts [20]. The biomass concentration (C_x) was calculated and expressed in g/L of the culture [19].

2.3. Experimental Setup

Experiments were performed from August to December 2019 in "raceway" open ponds located in the ITC facilities in the southeast region of the Gran Canaria Island (27°49′ N, 15°25′ E), located on the Atlantic coast with a semiarid, subtropical climate, an abundance of wind and plenty of sunlight. *T. striata* was cultivated semi continuously in each of the three systems: (1) 80 m^2 open pond located inside a greenhouse (RW80in) for 32 days; (2) 76 m^2 open pond located outdoors (RW76out) for 43 days, and finally the *T. striata* culture was scaled up to a (3) 300 m^2 open pond (RW300out) outdoors, cultivated semi-continuously for 28 days. The culture depth in RW80in and RW76out was set to d = 10 cm and d = 10.5 cm, respectively, in order to achieve an identical operational culture volume of 8000 L. The culture depth in RW300out was set to d = 15 cm, with an operational culture volume of 45,000 L. For the purpose of the inoculation and eventual backup of the biggest system (RW300out), another 76 m^2 pond (RW76out2) located in close proximity to RW76out was started one week prior to RW300out inoculation, in order to be used as the inoculum for the biggest pond, and afterwards this was maintained for 19 days as the backup in case of culture crash in RW300out, which did not occur until the end of the experiment. The two 8000 L ponds, RW80in and RW76out, were initially inoculated with 250 L (3.1% inoculum) of the 6-day-old *T. striata* culture, with C_x = 0.70 g/L. RW76out2 was inoculated with 500 L (6.2% inoculum) of culture from RW76out with C_x = 0.73 g/L, one week before its use as an inoculum for the biggest system (RW300out). The biggest system (RW300out, 45,000 L) was inoculated with 7500 L of culture from RW76out and 75,000 L of culture from RW76out2, which resulted in an initial biomass concentration in RW300out of C_x = 0.47 g/L. A high inoculum volume (33%) was used to avoid a possible early culture crash and to prevent the prolonged lag phase in such a large culture volume (45,000 L). The culture growth rate (μ, day^{-1}) for each open pond was calculated based on the initial biomass concentration growth data prior to the first harvest and the start of the semi-continuous cultivation, as explained in [21]. Ambient

temperature and solar irradiation per horizontal surface were measured constantly by Thies Clima 4.3350.10.000 and SunTracker: KippZonen Solys2 probes, and acquired by DataTaker DT-85. The temperature and daily solar irradiation energy data for the culturing period are presented in Appendix A (Figures A1 and A2). Cultures in the open ponds were sampled once a day around 10:00 AM to measure pH, salinity, OD at 750 nm, Q_y and biomass concentration. Each culture was also monitored daily under a light microscope (Leica DMi1, magnification ×40), to assess any eventual contaminations.

Open ponds were supplied constantly during the daytime (12 h/day) with 1 L/min of pure CO_2 in RW80in and RW76out and 3 L/min of pure CO_2 in RW300out. The pH was maintained in the physiological range (pH = 7.0 ± 0.8) through simultaneous CO_2 addition (which acidifies culture) and gradual nitrate assimilation by active algal culture (a process that consumes protons from the culture medium and maintains equilibrium of pH) [22,23]. Temperature inside the greenhouse was prevented from increasing above 30 °C via multiple industrial fan systems mounted on the main greenhouse wall. Apart from that, culture temperatures inside or outside the greenhouse were not externally controlled. Evaporation was measured based on the salinity changes and compared to initial salinities, then corrected daily by adding filtered fresh water. Each culture was circulated by an eight-blade paddle wheel, which provided superficial fluid velocity of 75 cm/s at 23 rpm in RW80in and RW76out and a fluid velocity of 47 cm/s at 20 rpm in the RW300out pond.

2.4. Semi-Continuous Pond Operation, Definitions and Calculations

The biomass concentration range that allowed us to select the initial set-point for starting harvest was determined using the well-established empirical relationship between the culture depth (d = 10 cm in RW80in and 10.5 cm in RW76out, respectively, and d = 15 cm in the 45,000 L pond) and the maximal biomass concentration of algae ($C_{x,max}$) that can be achieved in the open pond [10]:

$$C_{x,\,max} = \frac{9000}{d} \approx C_{x,setpoint}\ (\mathrm{mg_{DW}/L}) \quad (1)$$

The equation gives C_{max} in $\mathrm{mg_{DW}/L}$ if the pond depth is expressed in cm. Harvesting was performed semi-continuously in RW80in at an initial biomass concentration set-point of 0.5 g/L, which was increased from the 3rd harvest to 0.7 g/L. The biomass concentration set-point for harvest was 0.7 g/L in RW76out. The volume of harvested culture was determined by the minimal culture depth at the end of the process, which was set to 2 cm by the position of the inlet tube for the harvest pump. The evaporation was also accounted for by the salinity measurements. The harvested biomass was processed by centrifugation (Alfalaval VPNX 510SFD-34G), after which the open pond was refilled with fresh medium to an 8000 L total volume. The biomass productivity of each harvest was calculated by multiplying the total harvested volume and biomass concentration at the time of harvest:

$$\mathrm{P_{harvest}} = \mathrm{V_{harvest}} \cdot \mathrm{C_x} \quad (2)$$

This value (in $\mathrm{g_{DW}}$ or $\mathrm{kg_{DW}}$) can be considered as the maximal possible biomass productivity, and is contained in the harvested culture volume.

The biggest pond, RW300out, was harvested 8 times over the 28 days of semi-continuous cultivation, with the difference that we could measure the exact volume of harvested culture directly at the harvesting pump outlet in this pond. The biomass concentration set-point for harvest was set to 0.7 g/L. The biomass from RW300out was centrifuged, collected as paste and weighed on an industrial scale after each harvest. The water content of the biomass from RW300out was determined by drying a known amount of algal paste in Al trays overnight at 105 °C. The concentration factor of the centrifugation was determined using these data, comparing them to the harvested volume and biomass

concentration, calculated as the ratio of the dry weight in the algal paste to the biomass concentration of the initial culture:

$$CF = \frac{10(100 - \%\ water)}{C_x} \quad (3)$$

Daily horizontal solar irradiation energy ($I_{ground,daily}$, MJ/m^2/day) was calculated for each day of the experiment, based on the horizontal irradiation energies (W/m^2) recorded every minute by the SunTracker: KippZonen Solys2 system as an average value for that period. The recorded values for each day were summed up and multiplied by 60 s:

$$I_{ground,\ daily} = 60 \cdot \sum I_{ground} \quad (4)$$

The photosynthetic efficiency (PE, %) was calculated for a certain period of semi-continuous growth based on the combustion energy of the harvested biomass and the total irradiance on the pond surface, according to the equation [24]:

$$PE_{sunlight}(\%) = \frac{\Delta H_c^o \cdot Pharvest}{A \cdot \sum\ I_{ground,\ daily}} \cdot 100 \quad (5)$$

where $P_{harvest}$ is the biomass productivity of a harvest (g_{DW});
ΔH_c^o is the combustion enthalpy of algal biomass (22.5 kJ/g) [24];
A is the open pond illuminated surface (m^2).

The photosynthetic efficiency (%) of the indoor RW80in (8000 L) pond was calculated by multiplying the horizontal sunlight irradiation energy by 0.72 (see Figure A3, Appendix A). The degree to which the transparent roof of the greenhouse attenuates sunlight was calculated based on the light intensity reduction compared to the initial sunlight. Light intensities were measured with a Hansatech Quantitherm Lightmeter (Hansatech Instruments, Norfolk, UK outside the greenhouse and in seven different spots inside the greenhouse during August and September 2019. The data were averaged and presented as a percentage (%) of the outdoor sunlight intensity (Figure A3, Appendix A).

2.5. Determination of FA Profile and Heavy Metal Content in Algal Biomass

Various biomass samples harvested from RW80in and RW76out were freeze-dried (Lyobeta 6PL Telstar, freeze drier) and then acid-digested in Milestone Ethos Easy microwave digester, and finally used to determine heavy metal composition using ICP-OES (AVIO 500 Perkin Elmer).

Various biomass samples harvested from RW80in, RW76out and RW300out were freeze-dried and used to determine protein, carbohydrate, lipid and ash contents (gravimetrically), as well as the fatty acid profile, by GC-FID. Proximate composition was determined in three replicates to estimate the protein, lipids, carbohydrates, ash and water contents in the algal biomass by following standard procedures (AOAC, 2000, [25]). Protein content was determined using the Kjeldahl method [26], while crude lipid was quantified according to [27]. Water content was determined after drying the samples in an oven at 105 °C until reaching a constant weight, and ash content was assessed by combustion in a furnace at 550 °C for 12 h. Total carbohydrates were quantified by the difference in total algal biomass minus the content of all previous components (i.e., protein, lipids, ash, and water contents) [28].

2.6. Statistical Treatment of the Data

Statistical analysis was performed using Past3 software (Paleontological Statistics Software Package for Education and Data Analysis) [29]. Statistical differences between the temperature regime and solar irradiance in different cultivations, the biomass production in different ponds, and the biomass composition and metal contents of different samples were tested as follows: normality of the data was tested using Shapiro–Wilk and Anderson–

Darling tests; homogeneity of variance was tested by Levene's test; comparisons between groups were performed using (1) ANOVA test followed by Tukey's pairwise test for normally distributed data, or (2) Kruskal–Wallis test for equal medians followed by the Man–Whitney unpaired test when a normal distribution of the data could not be assumed. The significance level was set to 0.05.

3. Results and Discussion

3.1. Biomass Productivity during Long Term Cultivations in Various Ponds

There were, in total, seven harvests in 32 days for RW80in, with 80% of the volume harvested and replaced by fresh medium. In RW76out, a total of 11 harvests were performed during 43 days of cultivation, with 81% of the total volume harvested and replaced with fresh medium. The harvesting frequency was approximately two harvests per week for both 8000 L systems (Figure 1). The biomass concentration set-point for the harvest was set to 0.7 g/L in RW76out and RW80in. The biggest pond, RW300out, was harvested eight times in 28 days of semi-continuous cultivation, with the difference that we could measure the exact volume of harvested culture directly at the harvesting pump outlet in this pond. The biomass concentration set-point for harvests was 0.7 g/L, and the initial harvest volume was 18% (8100 L). After the first harvest, the biomass concentration excessively increased over the weekend, so the next harvest was 38% of the total volume (17,100 L). From this point onwards (2-Dec), the harvest volume was set to 22% (10,000 L) and this was maintained until the end of the experiment (six more harvests). At the same time, the medium was changed to 2f (3.52 mM of nitrate) with the addition of 1mM of urea, to prevent eventual N limitation. This enhanced culture medium was used in RW300out from the second harvest until the end of cultivation, with a total N concentration of 5.52 mM, which is sufficient to support a biomass concentration of 0.98 g/L, based on the assumption of 7.9% N in the dry algal biomass [16,17]. In order to maintain biomass production in the biggest pond (RW300out,) we wanted to prevent the limitation of N during long-term cultivation, as it is known that *Tetraselmis* sp. significantly decreases growth rate during N starvation, and it accumulates carbohydrates, thus reducing proteins and lipids [12], which suggests *T. striata* is not a lipid-accumulating species like other oleaginous species, such as *Nannochloropsis* sp. [30–32]. Nitrogen is one of the most important nutrients for growth, since it is a constituent of all structural and functional proteins, such as peptides, enzymes, chlorophylls, energy transfer molecules, and genetic materials in algal cell [12]. *Tetraselmis* spp., similar to most microalgae, are able to utilize various forms of nitrogen, including nitrate, nitrite, ammonium, and organic nitrogen sources such as urea [12]. The addition of 1 mM (60 mg/L) of urea to the *T. striata* culture medium (apart from providing an additional 2 mM of N for cell division) effectively inhibited the appearance of grazers, and neither amoeba nor ciliates were detected after urea addition in any open pond throughout the long-term cultivations (based on the daily checking by the optical microscopy). This is in in accordance with the report of Mendez and Uribe (2012), who found that the application of 60 mg/L (1 mM) of urea to the outdoor mass culture of contaminated *Arthrospira* sp. completely inhibited grazing, while at the same time it did not negatively affect the growth of the culture, but rather promoted the rapid recovery of algal density to levels that obtained prior to infestation [15]. Similarly to what was reported by [15], we can confirm that urea provided an economical source of N, and was simultaneously a very effective means of controlling predators in cultures of *T. striata*. A high resistance to contamination in long-term mass cultures of *Tetraselmis* sp. has already been reported [3]. A recent study also used a modified medium containing urea, phosphate, Mg, Fe and bicarbonate in seawater to grow *T. striata* in a 2000 L open pond [1]. The results showed that *T. striata* had a higher areal biomass productivity, specific growth rate and lipid content in modified medium compared to the more expensive f medium, which agrees with our findings that this strain can be economically cultivated in simple inorganic seawater media using industrial-grade chemicals [1]. Effective grazer control via the addition of a cheap and readily assimilated N source such as urea is the apparent advantage of using *Tetraselmis* sp. compared to more

commonly used industrial microalgal species such as *Chlorella vulgaris* and *Haematococcus pluvialis*, which are susceptible to predators and parasites in industrial settings [33–35]. In our study, the average paste humidity obtained by centrifugation (Alfalaval VPNX 510SFD-34G) was 85.3 ± 0.65% and 86.8 ± 0.18% for RW76out and RW300out, respectively. The concentration factors upon centrifugation were 207.9 ± 0.21 and 231.3 ± 0.09 for RW76out and RW300out, respectively (see Table A1, Appendix A).

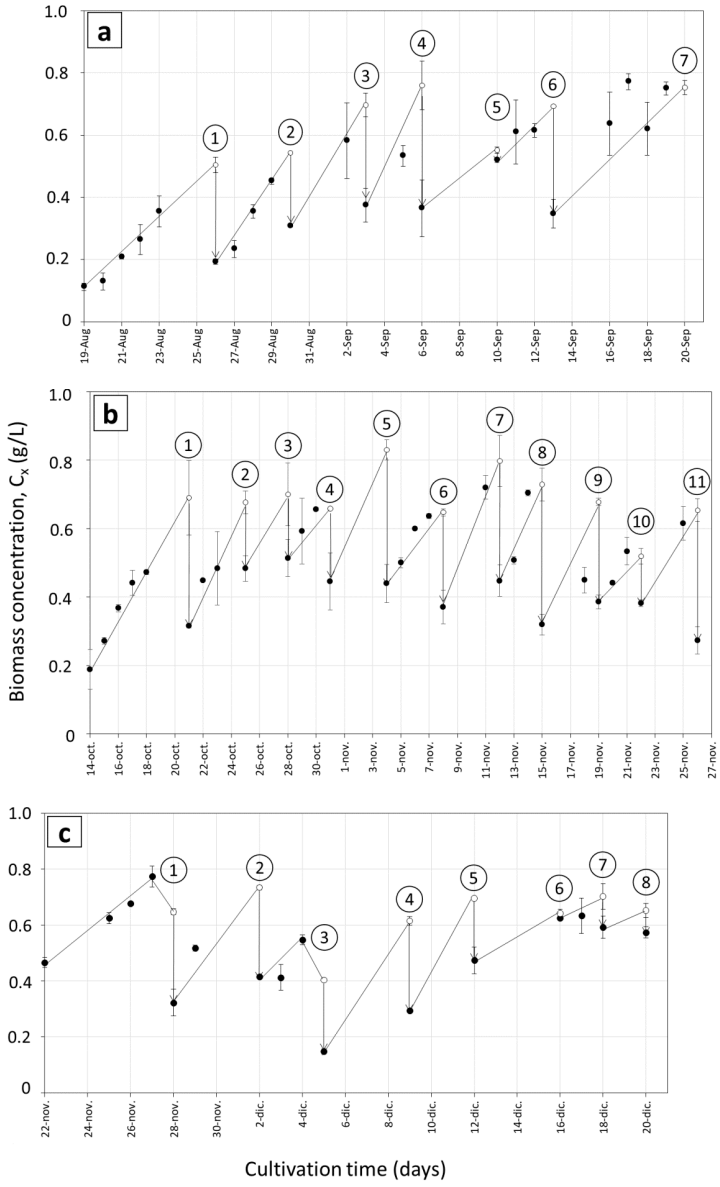

Figure 1. Biomass concentration (C_x, g/L) of the *T. striata* cultures over the time-course of the semi-continuous cultivation (**a**) in the 8000 L raceway pond inside the greenhouse (RW80in), (**b**) in the 8000 L raceway pond outdoors (RW76out), and (**c**) in the 45,000 L raceway pond outdoors (RW300out). Each harvest is indicated by a number tag. There were in total 7 harvests in a 32-day period for RW80in, 11 harvests in 43 days of cultivation for RW76out, while RW300out was harvested 8 times in 28 days of semi-continuous cultivation.

The long-term biomass productivities of microalgae in large-scale open ponds vary depending on the literature source—from 30 t/ha/year in Spain [36] to 91 t/ha/year in Australia [37]—although it normally rarely exceeds 40 t/ha/year [38]. Biomass productivities for the cultivation periods are presented in Table 1. If we simply interpolate this data to year-round operation and per 1 hectare of the open ponds (300 days × 10,000 m^2

of open pond surface), we can obtain an estimation of the annual biomass productivities for *T. striata* based on the three tested systems: 24.66 ± 0.53 kg_{DW} in 32 days (or 28.9 t/ha/year) for RW80in, 42.32 ± 0.81 kg_{DW} in 43 days (or 38.8 t/ha/year) for RW76out and 54.9 ± 0.58 kg_{DW} in 28 days (or 19.6 t/ha/year) for RW300out. Based on these data, we can conclude that the estimated annual biomass production decreases with pond size, becoming more realistic and similar to previous reports [39]. The average value of annual biomass productivity per ha for these three systems is 28.9 t/ha/year, which agrees with the reported annual productivity of 31 t/ha/year for the Indian native strain *T. striata* BBRR1 cultivated in 10 m^2 open ponds [1], and the yield of 36 t/ha/year of *T. suecica* produced in a large-scale flat-panel PBR [40,41]. The annual biomass production of *P. tricornutum* in open ponds was 41.5 t/ha/year for The Netherlands, and 63.7 t/ha/year for Algeria [42]. Our results confirm previous findings that *Tetraselmis* can be scaled-up to industrial PBRs [8,43]. *Tetraselmis* sp. was successfully scaled up to 100 m^3 closed tubular PBRs during a 60-day cultivation, and in that study, the volumetric and areal productivities obtained in semi-continuous cultivation were 0.08 g/L/d and 20.3 $g/m^2/d$, respectively, with the biomass containing 9–10% lipids in dry weight [3].

Table 1. Biomass productivities P_{DW} (kg_{DW}) per harvest during long-term semi-continuous cultivations in three different raceways: RW80in—8000 L pond inside greenhouse, RW76out—8000 L pond outdoors, RW300out—45,000 L pond outdoors. Initial growth rates in the batch mode (μ (day^{-1})) were calculated based on the exponential growth during the initial eight days of batch culture, prior to the first harvest: μ = 0.21 ± 0.021 day^{-1} in RW80in, 0.19 ± 0.068 day^{-1} in RW76out and 0.10 ± 0.018 day^{-1} in RW300out.

RW80in (8000 L–80 m^2)		**RW76out (8000 L–76 m^2)**		**RW300out (45,000 L–300 m^2)**		
Harvest	**$P_{DW, max}$ (kg_{DW})**	**Harvest**	**$P_{DW, max}$ (kg_{DW})**	**Harvest**	**$P_{DW, max}$ (kg_{DW})**	**Paste (kg)**
26-Aug	2.61 ± 0.13	21-Oct	3.72 ± 0.28	28-Nov	5.24 ± 0.09	18.0
30-Aug	2.71 ± 0.01	25-Oct	3.47 ± 0.17	2-Dec	12.56 ± 0.13	51.0
3-Sep	3.69 ± 0.20	28-Oct	3.81 ± 0.50	5-Dec	4.04 ± 0.02	11.4
6-Sep	4.38 ± 0.45	31-Oct	4.13 ± 0.01	9-Dec	6.15 ± 0.16	17.3
10-Sep	2.89 ± 0.05	4-Nov	4.03 ± 0.14	12-Dec	6.96 ± 0.02	21.4
13-Sep	3.95 ± 0.02	8-Nov	3.88 ± 0.06	16-Dec	6.42 ± 0.14	18.0
20-Sep	4.44 ± 0.13	12-Nov	3.93 ± 0.37	18-Dec	7.03 ± 0.46	31.2
Total (kg_{DW})	24.66 ± 0.53	15-Nov	4.30 ± 0.28	20-Dec	6.53 ± 0.25	24.8
		19-Nov	3.99 ± 0.06	Total (kg_{DW})	54.9 ± 0.58	193.1
		22-Nov	3.07 ± 0.13			
		26-Nov	3.99 ± 0.20			
		Total (kg_{DW})	42.32 ± 0.81			

3.2. Growth Rates and Photosynthetic Efficiency of Open Pond Cultures

The growth rates (μ, day^{-1}) of the cultures were very similar in two smaller systems (0.21 ± 0.021 day^{-1} in RW80in and 0.19 ± 0.068 day^{-1} in RW76out), while the growth rate was two times lower in the 45,000 L system (0.10 ± 0.018 day^{-1} in RW300out), which can be explained by the higher inoculum volume (33% in RW300out vs. 3.1% in RW76out) and the lower amount of light available in the biggest system, as the culture depth was increased to 15 cm in RW300out, which would directly influence culture performance in an open pond [10]. It was reported that *T. striata* had a maximal growth rate of 0.45 day^{-1} in a 10 m^2 (2000 L) open pond [1], which seems to confirm that the growth rate will decrease when scaling up, as a larger scale reduces liquid circulation, mixing efficiency, light availability and gas exchange [10]. However, *Tetraselmis* sp. cultivated in an aerated, small-scale vessel

(100 mL) placed inside the growth chamber had growth rates in the 0.29–0.31 day^{-1} range, which is much closer to the values obtained in this study [44].

Photosynthetic efficiency PE (%) is the essential parameter for solar-driven cultivations of microalgae, and it indicates the efficiency of light utilization by algae in a given photobioreactor. The maximum theoretical PE is as high as 12.4%, and can be calculated based on the chemical energy stored in the fixed mole of CO_2 reduced to glucose and the average energy of quantum requirement (8 moles of red photons) [24]. In reality, microalgae can conserve only small part of the available solar energy, and photosynthetic efficiency in microalgal outdoor systems rarely exceeds 5–6% [45].

PE (%) in this study was calculated based on the combustion enthalpy of the obtained biomass and total daily irradiances, as explained in the Materials and Methods section. The cumulative PE, calculated based on total produced biomass and total impinging photons of sunlight for the entire cultivation period, are 1.45 ± 0.03% in RW80in, 1.95 ± 0.04% in RW76out and 1.10 ± 0.01% in RW300out (Table 2). These values are much lower than the PE values reported for large-scale cultures of *Tetraselmis* sp. (2.38 ± 0.27 and 3.35 ± 0.19% for 30,000 L and 100,000 L tubular PBRs, respectively) [3], which is also probably due to the greater light penetration depth, higher surface to volume ratio, shorter optical path and higher productivity in tubular systems than in open ponds [45]. Nevertheless, the maximum PE values achieved in this study were 3.52 ± 0.36% in RW80in during the first week of September and 3.49 ± 0.23% for RW76out for the second week of November (see Table 2). These are very similar to the 3.35 ± 0.19% PE achieved in a 100 m^3 tubular PBR [3], which demonstrates the high biological and physiological potential and productivity of the selected *Tetraselmis* strain. PE values of 2.09% in The Netherlands and 1.56% in Algeria were reported for the long-term cultivation of *P. tricornutum* in large-scale open ponds [42], while values of PE = 1.5% are generally obtained for *A. platensis* cultivated in open raceway ponds [46]. The authors concluded that the overall PE depends on both the algae species and the location, while cultures in countries with a moderate climate use the available light more efficiently [42]. The average PE for an 8000 L indoor pond (1.45%) obtained in this study is very close to the 1.5% PE for large-scale open ponds in general [45], and the estimated 1.56% PE for *P. tricornutum* in open ponds in Algeria [42].

Measurements of chlorophyll fluorescence, and especially quantum yield Q_y, are fast and sensitive ways to determine whether the photosynthetic efficiency of the culture is affected by chemical, physiological or culturing parameters [47]. Healthy algal cultures have a Q_y in the 0.7–0.8 range, and any quantum yield decrease is a signal of a stressed or otherwise negatively affected culture [48]. Daily measurements of quantum yields for the cultures in two outdoor ponds (Figure A4, Appendix A) showed no evidence of any decrease in the photosynthetic performance of the cells during long-term semi-continuous growth, as the maximum photosynthetic efficiency of PSII was Q_y = 0.71 ± 0.06 and 0.70 ± 0.03 for RW76out and RW300out, respectively. The lowest Q_y values (0.53–0.62) were only detected at the start of the cultivations and in freshly harvested cultures (Figure A4, Appendix A) recently supplied with fresh medium, which were likely stressed due to the photosaturation in the thin culture that was suddenly exposed to full sunlight [24].

Table 2. Photosynthetic efficiencies (PE, %) of *T. striata* cultures, calculated as explained in the Materials and Methods section, based on the biomass productivity per harvest and the daily horizontal solar irradiance energy received per reactor surface, during long-term semi-continuous cultivations in three different raceways: RW80in—80 m^2 pond inside greenhouse, RW76out—76 m^2 pond outdoors, RW300out—300 m^2 pond outdoors. Cumulative PE (%) for each cultivation was calculated based on the total biomass productivity and the total light energy received per pond surface during the time-course of the entire experiment. Solar irradiation energies for RW80in inside the greenhouse were corrected by including the light intensity attenuation of the greenhouse roof (72% of the solar light energy is passed through the roof, on average). There is no statistical difference between PE (%) values in smaller systems, yet there is a statistical difference ($p < 0.05$) between solar PE values for the RW76out and RW300out ponds: (RW80in) cumulative 1.45, mean 1.69, median 1.40%; (RW76out) cumulative 1.95, mean 2.17, median 2.03%; (RW300out) cumulative 1.10, mean 1.22, median 1.11%.

RW80in (8000 L–80 m^2)		**RW76out (8000 L–76 m^2)**		**RW300out (45,000 L–300 m^2)**	
Harvest	**PE (%)**	**Harvest**	**PE (%)**	**Harvest**	**PE (%)**
26-Aug	0.65 ± 0.03	21-Oct	0.84 ± 0.06	28-Nov	0.41 ± 0.01
30-Aug	1.36 ± 0.00	25-Oct	1.82 ± 0.09	2-Dec	1.83 ± 0.02
3-Sep	1.66 ± 0.09	28-Oct	3.18 ± 0.42	5-Dec	0.84 ± 0.00
6-Sep	3.52 ± 0.36	31-Oct	2.51 ± 0.00	9-Dec	0.91 ± 0.02
10-Sep	1.36 ± 0.02	4-Nov	1.76 ± 0.06	12-Dec	1.29 ± 0.00
13-Sep	2.33 ± 0.01	8-Nov	1.88 ± 0.03	16-Dec	1.12 ± 0.02
20-Sep	1.20 ± 0.04	12-Nov	2.01 ± 0.19	18-Dec	1.84 ± 0.12
Cumulative PE, %	1.45 ± 0.03	15-Nov	3.49 ± 0.23	20-Dec	1.76 ± 0.07
		19-Nov	2.34 ± 0.04	Cumulative PE, %	1.10 ± 0.01
		22-Nov	2.06 ± 0.09		
		26-Nov	2.20 ± 0.11		
		Cumulative PE, %	1.95 ± 0.04		

3.3. Biomass Quality, FA Profile and Heavy Metal Content

The micrioalgal biomass of the species from the *Tetraselmis* genus has many important nutritional properties, due to its interesting biochemical composition and different bioactive properties, including antioxidant, metal-chelating, neuroprotective, cell-repairing, and cytotoxic activities [49]. On December 2017, the EU Commission updated the European list of novel foods in accordance with Regulation (EU) 2017/2470, to include dried biomass from microalga *Tetraselmis chuii* as a novel food for human consumption (EC implementing regulation (EU) 2017/2470) [9]. According to this decision, *T. chuii* biomass can be marketed as human food as long as it complies with the following regulations: it has to be cultivated in sterilized seawater medium in closed systems, and it has to be made of 35–40% proteins, 30–32% carbohydrates, 5–8% lipids and 14–16% ash, under ≤7% humidity [9]. Figure 2 shows the basic biomass constituents (proteins, carbohydrates, lipids and ash in % of biomass dry weight) of samples from each cultivation system employed in our study. The average content of the *T. striata* biomass was 35.9% proteins, 19.7% carbohydrates, 5.5% lipids, 38.9% ash. *Tetraselmis* sp. cultivated in synthetic seawater (with 10 g/L salt) had a biomass composition with similar protein and lipid contents as our biomass (41.1 ± 0.09% proteins, 5.58 ± 0.06% lipids), but much more carbohydrate (45.1 ± 0.27%) and a lower ash content (8.22 ± 0.3%) [43]. *Tetraselmis* species usually have high protein contents; *T. chuii* used as shrimp larvae feed contains 36.86 ± 1.93% proteins, 20.83 ± 0.65% carbohydrates and 11.74 ± 0.49% lipids [6], while *T. suecica* grown under N-sufficient conditions in outdoor flat panel systems contains 50% proteins, 15% carbohydrates and 30% lipids [50]. However, the same species (*T. suecica*) grown in half-liter bubbled columns as a non-axenic culture had the following biomass composition: 17–18% proteins, 37–43% carbohydrates, 16–18% lipids and 17–18% ash [13]. This would suggest that culture conditions are more decisive in

determining biochemical composition than interspecific differences within the *Tetraselmis* genus. Nutrient limitation can have significant effects on algal biomass composition [1–4]. The lack of free available nitrogen in the culture medium eventually stops protein synthesis and triggers the accumulation of lipids and/or carbohydrates, depending on the algal species [5]. It was also reported that *Tetraselmis* sp. biomass, produced in 100 m^3 closed PBRs, contained: 31.2% proteins, 18.1% carbohydrates, 7.04% lipids, 15.2% ash and 24.6% dietary fibers [49]. It was reported that *Tetraselmis* sp. biomass had approximately 24% proteins, 42% carbohydrates, and 15% lipids, calculated per dry weight [12]. The ash content in our study varied from 32.8 ± 0.14% in RW80in to 38.8–45.5% in RW300out, depending on the harvest (Figure 2). It is apparent that the ash content in the biomass was higher in outdoor conditions and in larger cultures. The higher ash content in our study (38.9% in average) compared with the 15.2% reported in closed 100 m^3 PBRs [3,49] can be explained by the high exposure of open outdoor ponds to dust and particles. The possible solution to this issue is to include one additional step into the down streaming process at our facility of washing the fresh paste taken from the centrifuge. The improvement in biomass quality by the reduction in ash content by such a washing procedure will be a part of future investigations, and merits our close attention. Variability in environmental factors, such as light availability and temperature, may have played a role in determining differences among different cultivations. Even if seasonal differences are not that as drastic in Gran Canaria as they would be in continental Europe, both daily horizontal solar irradiation energy (Figure A1, Appendix A) and daily average, maximal and minimal temperatures (Figure A2, Appendix A) were significantly different among the three cultivation periods: (a) 19-Aug to 20-Sep; (b) 14-Oct to 26-Nov, and (c) 22-Nov to 20-Dec. Such differences may have been responsible for changes in the biochemical profile between the biomass from different cultures (Figure 2).

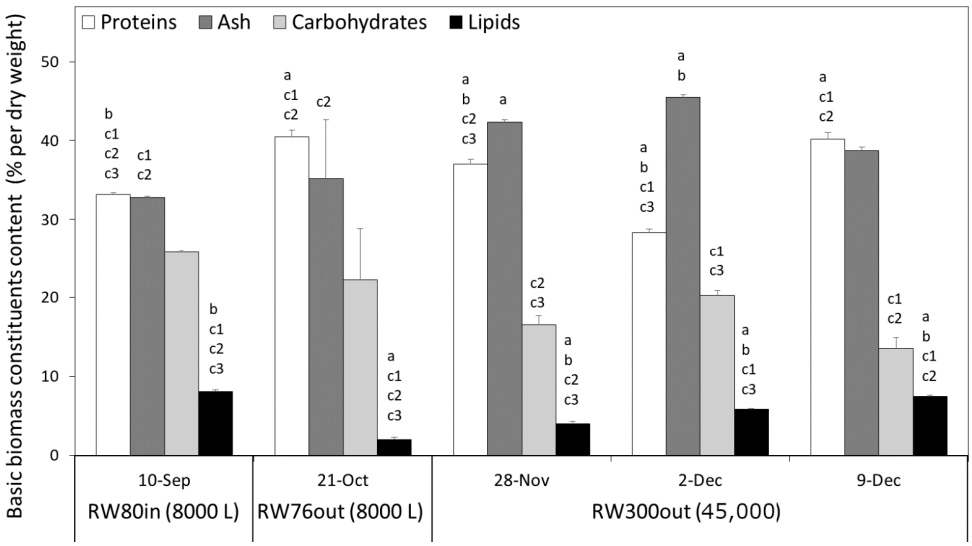

Figure 2. Content of the basic biomass constituents (proteins, carbohydrates, lipids and ash in % of the biomass dry weight) for samples from each cultivation system (RW80in–8000 L pond inside greenhouse, RW76out–8000 L pond outdoors, RW300out–45,000 L pond outdoors) during the long-term semi-continuous cultivation. Letters above bars represent (for each biomass constituent): a = significative difference in RW80in biomass harvested on 10-Sep, b = significative difference in RW76out biomass harvested on 21-Oct, c1 = significative difference in RW300out biomass harvested on 28-Nov, c2 = significative difference in RW300out biomass harvested on 2-Dec, c3 = significative difference in RW300out biomass harvested on 9-Dec. All data are presented as the average of three technical replicates with corresponding standard deviation (±SD). Legend: white bars—proteins; dark gray bars—ash; light gray bars—carbohydrates; black bar—lipids.

The fatty acid profile of the most common microalgae species predominantly contains palmitic (C16:0), oleic (C18:1) and linoleic acid (C18:2) [18,50–52], which was the case in

this study as well (Table 3). From the FA profiles in Table 3, we can also conclude that there are minor differences in the FA profiles among three cultivation systems ($p > 0.05$) and harvests, with the exception that the indoor culture had more oleic (21.1%) and less linolenic (5.9%) FA than outdoor cultures. According to our results, the major fatty acids (that comprise more than half of the total FA) in *T. striata* are: 19.7 ± 2.4% palmitic (C16:0), 15.5 ± 4.1% oleic (C18:1), 5.2 ± 2.0% linoleic (C18:2), and 14.4 ± 5.7% linolenic (C18:3) FA on average, expressed in % of total FA. This agrees with previous reports that the major fatty acids in *Tetraselmis* sp. are palmitic (C16:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) FA [1,12], with the difference that we found more EPA (20:5n-3) than linoleic acid in *T. striata* biomass (6.3 ±1.5% EPA compared to 5.2 ± 2.0% linoleic FA). It was reported that palmitic (C16:0), oleic (C18:1) and linoleic (C18:2) acids are the major FA in transmethylated lipids from *Tetraselmis* sp., representing approximately 75% of the total fatty acids [44]. The authors further reported that palmitoleic (C16:1) and hexadecatrienoic (C16:3) acids form another 10% of the total FA, and finally only minor levels of hexadecadienoic (C16:2), linolenic (C18:3) and eicosapentaenoic (C20:5n-3, EPA) acids were detected [44]. Polyunsaturated FA, particularly EPA (20:5n-3) and DHA (22:6n-3), are considered FA of major importance in the nutritional composition of an algal species that has to be used as food for marine organisms [53]. The average PUFA content in total FA, in our study, was 6.3 ± 1.5% for 20:5n-3 (EPA) and 3.1 ± 1.1% for 22:6n-3 (DHA). Similarly, *T. chuii* cultivated in optimized f/2 medium was reported to have 7.4% EPA and 6.6% DHA, as a % of total FA [53]. The differences in EPA and DHA content, especially in the samples from large outdoor systems, can be attributed to changes in ambient temperature, which can affect the degree of saturation in the algal FA profile. It was reported that temperature increase can have a positive effect on algal FA saturation degree, which contradicts our findings, although the other authors tested a much broader temperature range (15–40 °C) than in our case (5 °C) [54]. It is also possible that the EPA and DHA content reductions in RW300out from 28-Nov to 9-Dec were related to the nutrient status of the culture, but as we did not monitor nutrient assimilation in the culture medium, any closer connection between unsaturated FA content and nutrient levels cannot be made. Three different strains of *Tetraselmis* were generally characterized by approx. 4% EPA in total FA, and very low contents of DHA [55]. The feeding of brine shrimp (*Artemia franciscana*) with *T. chui* biomass resulted in significant increases in protein, carbohydrate and lipid contents of brine shrimp, which was attributed to the favorable FA profile of the microalga [53]. *Tetraselmis* biomass was also found to be a suitable part of the *C. gigas* (Pacific oyster) larvae diet [55].

The biomass samples of *T. striata* obtained in this study from outdoor and indoor open ponds are free from As, Hg, and Pb, as these heavy metal contents were below the detection limit in all tested samples (Table A2, Appendix A). The indoor culture was also free from Cd, while the outdoor culture had only trace amounts of 54 ng/g_{DW} Cd. Based on these data, we can conclude that the As, Hg, Pb and Cd contents in tested samples comply with the EU directive and regulations for undesirable substances in animal feed (EC 32/2002 and EC 1869/2019), and also with the EU regulation for heavy metals in food supplements (EC 629/2008) [56–58].

This goes in accordance with previously reported data for *Tetraselmis* sp. biomass, produced in 100 m^3 closed PBRs that contained low amounts of Cd and no other heavy metals, but also no pathogenic bacteria, cyanotoxins, mycotoxins or polycyclic aromatic hydrocarbons, suggesting that this strain can be used for nutritional applications [49]. The heavy metal content data from this study are similar to those reported for *Tetraselmis* sp. grown in semi-continuous industrial tubular reactors, where authors found no As, Hg, or Pb, and only trace amounts of 200 ng/g_{DW} of Cd [49].

Table 3. Fatty acid profile of *Tetraselmis striata* biomass from various open ponds and harvests. Fatty acids content is presented as % of total FA based on GC-FID chromatogram for each biomass sample.

Harvest Date	**10-Sep**	**21-Oct**	**28-Nov**	**2-Dec**	**9-Dec**	
Fatty Acid (% of Total FA)	**RW80in**	**RW76out**	**RW300out**	**RW300out**	**RW300out**	**Average**
14:0	1.9	0.2	0.9	0.9	0.8	0.9 ± 0.6
16:0 (Palmitic)	20.4	16.8	18.4	19.6	23.2	19.7 ± 2.4
16:1n-7	2.0	2.3	1.2	1.2	1.0	1.6 ± 0.6
16:3n-1	3.6	12.7	8.7	7.3	8.0	8.1 ± 3.3
18:0	2.7	0.6	1.9	1.5	1.4	1.6 ± 0.8
18:1n-9 (Oleic)	21.1	18.8	12.1	13.3	12.3	15.5 ± 4.1
18:2n-6 (Linoleic)	8.2	6.1	3.6	4.3	3.7	5.2 ± 2.0
18:3n-3 (ALA)	5.9	22.1	14.7	15.1	14.5	14.4 ± 5.7
18:4n-3	2.3	4.0	5.8	4.4	4.5	4.2 ± 1.3
20:5n-3 (EPA)	5.9	5.3	8.7	6.8	4.8	6.3 ±1.5
22:6n-3 (DHA)	3.1	-	3.5	4.3	1.6	3.1 ± 1.1

It is obvious that long-term cultivation in outdoor conditions requires strains such as native *T. striata*, which is robust, resistant to grazers, and characterized by high resistance to environmental condition changes [59]. In the large-scale cultivation of microalgae, fast growth and resistance to high temperatures are important because they allow higher productivity and also reduce the risk of contaminations [60]. There are some indications that *Tetraselmis* sp. is readily flocculating in the later stationary phase, which is of importance to decreasing the cost of centrifugation [1,43,49]. In previous studies, biomass of *T. striata* was harvested from a 2000 L open pond by switching off the paddle wheel and letting the culture settle for 12 h, followed by the collection of flocculated pellets after the medium was drained off [1]. *Tetraselmis* sp. was also cultured semi-continuously in 2.5 m^3 tubular PBRs, for four days, and stored in a 1000 L harvesting tank for pre-concentration. Sedimentation for 24 h resulted in the removal of 93% of the culture medium in the form of a clear liquid, and the remaining culture was recovered as a highly concentrated biomass (19.5 g/L) and wet microalgal paste (272.7 g/L) [43]. The authors concluded that 24 h sedimentation as a pre-concentration step provides an effective recovery of 97% of the total biomass, significantly reducing the harvesting costs [43]. A culture medium with high salt concentration and/or based on seawater can increase the sedimentation rate because the ionic strength of saline solutions could affect the negative charges at the cell surface, and decrease the zeta potential of the algal cell wall [43]. There is an ongoing investigation in our facilities regarding the flocculation affinities of *T. striata* and the economics of this pre-concentration step compared to centrifugation, which we hope will result in another publication that will bring additional value to this study in the future.

4. Conclusions

Based on the results of this study, we can conclude that *T. striata* is a good candidate for large-scale biomass production on Gran Canaria Island. It is fast-growing and can be rapidly scaled up to very high culture volumes; it easily forms a healthy culture that maintains high photosynthetic activity during long-term cultivation; it provides stable biomass productivities in various seasons of the year; it is able to grow on economical sources of nitrogen (fertilizers, such as urea), which act simultaneously as very cheap and possibly effective grazer controls. Additionally, it is a good source of proteins, and has an FA profile abundant in some nutritionally important fatty acids, such as oleic, α-linolenic (ALA) and EPA, which encourages the use of *T. striata* biomass as feed and food. The high ash content (>35%) in the biomass obtained from outdoor open ponds can be resolved

in the future via the implementation of an additional washing step after centrifugation of the culture.

Author Contributions: Conceptualization, investigation and original draft preparation, Z.G.; writing—review and editing, B.B., M.V., F.G.; methodology, Z.G. and F.G.; methodology, P.A.C.J.d.A.; writing—review and editing and funding acquisition, E.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the following projects: SEIDI resolution No 8/19, SEIDIAMP resolution No 47/19, REBECA (Interreg MAC 2014-2020) MAC/1.1A/060, REBECA CCT (Interreg MAC 2014-2020) MAC2/1.1b/269, MEDREGALGAE resolution No 10/2019.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: We would like to thank Ana Marcos de los Ríos, Head of Production of Algalimento S.L company (Pozo Izquierdo, Gran Canaria, Spain) for kindly lending us part of their equipment for our experiments, including the 45,000 L open pond. We also would like to thank Antera Martel Quintana and Carlos Almeida from Spanish Bank of Algae (BEA, Telde, Gran Canaria, Spain) for the scientific support in strain characterization. Special thanks go to Sara Gonzalez, Emilio Rosario, María Macarena González Hernández and Antonio Suarez Vega from the Biotechnology department of ITC, for their hard work in the raceway maintenance and technical support in the greenhouse. We also would like to thank Deullanela Díaz Hernández from Renewable Energies Department of ITC for the assistance in meteorological data collection and processing.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

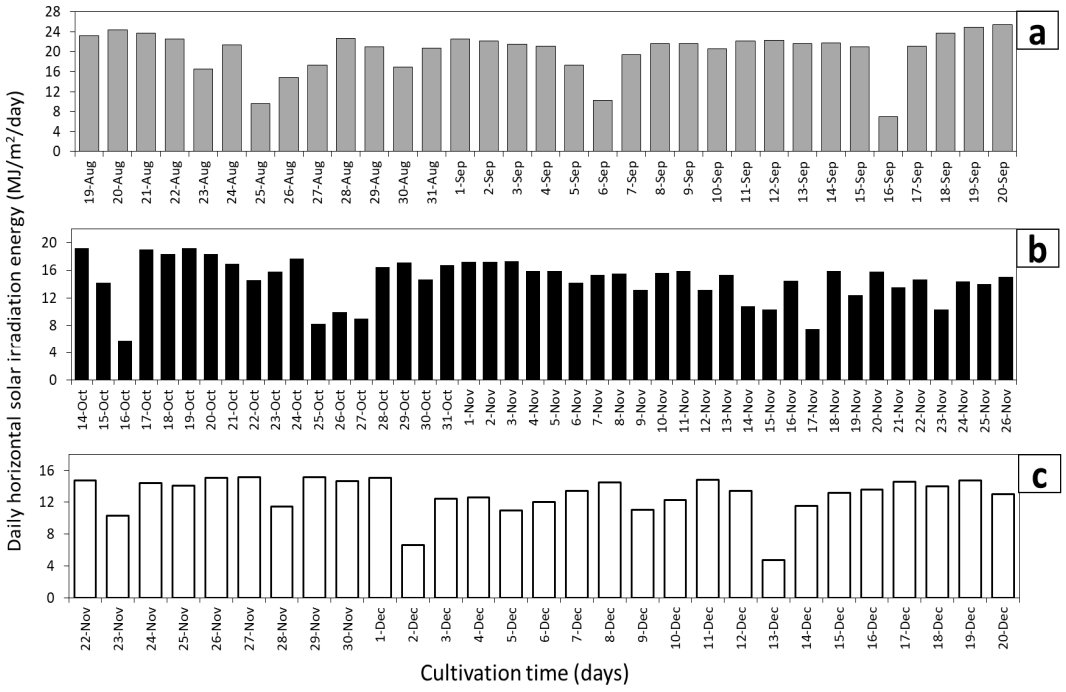

Figure A1. Daily horizontal solar irradiation energy ($I_{ground,\ daily}$, MJ/m^2/day) of the *T. striata* cultures throughout the time-course of the semi-continuous cultivation for the (**a**) 8000 L raceway pond inside the greenhouse (RW80in); (**b**) the 8000 L raceway pond outdoors (RW76out); (**c**) the 45,000 L raceway pond outdoors (RW300out). There are statistical differences ($p < 0.01$) between daily horizontal irradiances for the three cultivation periods: (**a**) 19-Aug to 20-Sep: mean 20.1, median 21.5 MJ/m^2/day; (**b**) 14-Oct to 26-Nov: mean 14.6, median 15.4 MJ/m^2/day; (**c**) 22-Nov to 20-Dec: mean 12.9, median 13.4 MJ/m^2/day.

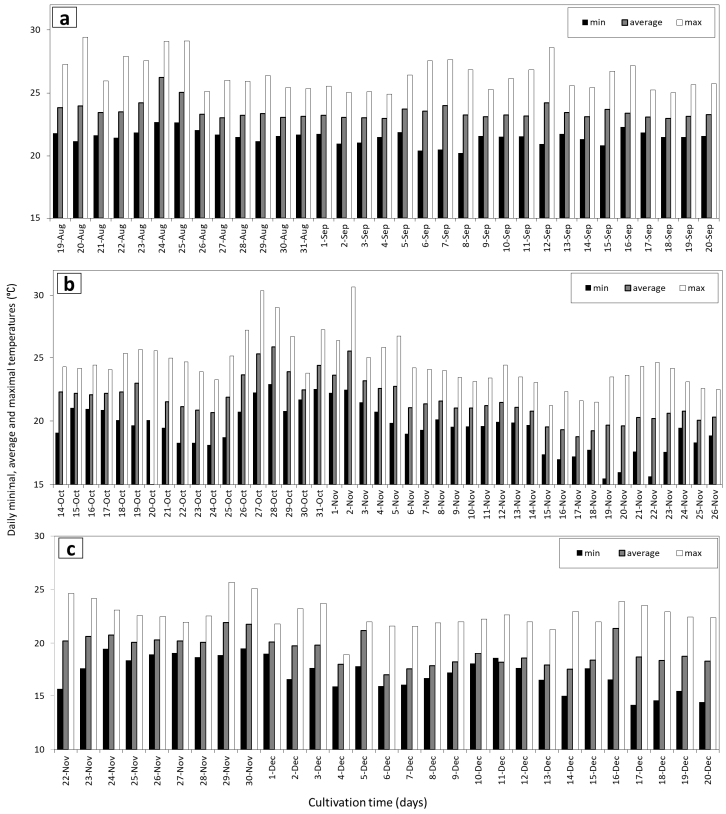

Figure A2. Daily average, maximal and minimal temperatures (°C) of the *T. striata* cultures throughout the time-course of the semi-continuous cultivation (**a**) for the 8000 L raceway pond inside the greenhouse (RW80in); (**b**) for the 8000 L raceway pond outdoors (RW76out); (**c**) for the 45,000 L raceway pond outdoors (RW300out). There are statistical differences ($p < 0.01$) between average daily temperatures for the three cultivation periods: (**a**) 19-Aug to 20-Sep: mean 23.5 °C, median 23.3 °C; (**b**) 14-Oct to 26-Nov: mean 21.2 °C, median 21.3 °C; (**c**) 22-Nov to 20-Dec: mean 19.3 °C, median 19.0 °C.

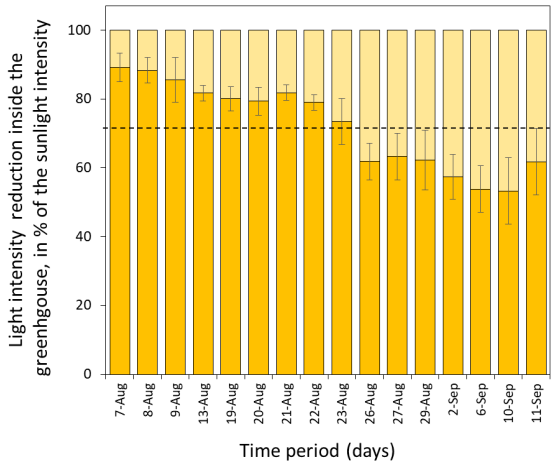

Figure A3. Sunlight irradiation reduction inside the greenhouse compared to the outdoor light irradiation, expressed in % of solar irradiation energy. Light irradiation was measured with a Hansatech Quantitherm Lightmeter (Hansatech Instruments, Norfolk, UK) outside the greenhouse and in seven different locations inside the greenhouse during August and September 2019. The data were calculated and are presented as % of the outdoor sunlight irradiation. The average light attenuation for the time period was such that 72% on average of daily sunlight irradiation was allowed to pass through the greenhouse roof. Legend: yellow bars—full sunlight; orange bars—light intensity inside the greenhouse; horizontal dashed line—72% of sunlight irradiation allowed to pass through the transparent greenhouse roof.

Table A1. Water content (paste humidity, %) and the concentration factor of the harvested wet algal paste after centrifugation (Alfalaval VPNX 510SFD-34G), compared to the biomass concentration of the harvested culture for: RW76out—8000 L pond outdoors; and RW300out—45,000 L pond outdoors.

RW76out (8000 L–76 m^2)			RW300out (45,000 L–300 m^2)		
Harvest	**Paste Humidity (% H_2O)**	**Conc. Factor**	**Harvest**	**Paste Humidity (% H_2O)**	**Conc. Factor**
21-Oct	83.5 ± 0.62	239.3 ± 0.16	28-Nov	87.5 ± 0.00	193.3 ± 0.02
25-Oct	87.5 ± 0.14	187.4 ± 0.05	2-Dec	87.5 ± 0.01	300.6 ± 0.02
31-Oct	85.1 ± 0.10	226.0 ± 0.00	5-Dec	86.8 ± 0.01	328.2 ± 0.01
4-Nov	80.0 ± 0.05	241.2 ± 0.04	9-Dec	83.3 ± 0.01	270.9 ± 0.03
8-Nov	87.9 ± 0.05	187.5 ± 0.02	12-Dec	86.4 ± 0.18	195.5 ± 0.00
12-Nov	83.2 ± 0.04	210.0 ± 0.09	16-Dec	88.2 ± 0.00	183.1 ± 0.02
15-Nov	87.8 ± 0.00	167.1 ± 0.07	18-Dec	89.6 ± 0.02	147.9 ± 0.07
19-Nov	88.0 ± 0.04	177.5 ± 0.02	20-Dec	84.9 ± 0.01	231.0 ± 0.04
26-Nov	84.5 ± 0.01	237.4 ± 0.05	-	-	-
Average	85.3 ± 0.65	207.9 ± 0.21	Average	86.8 ± 0.18	231.3 ± 0.09

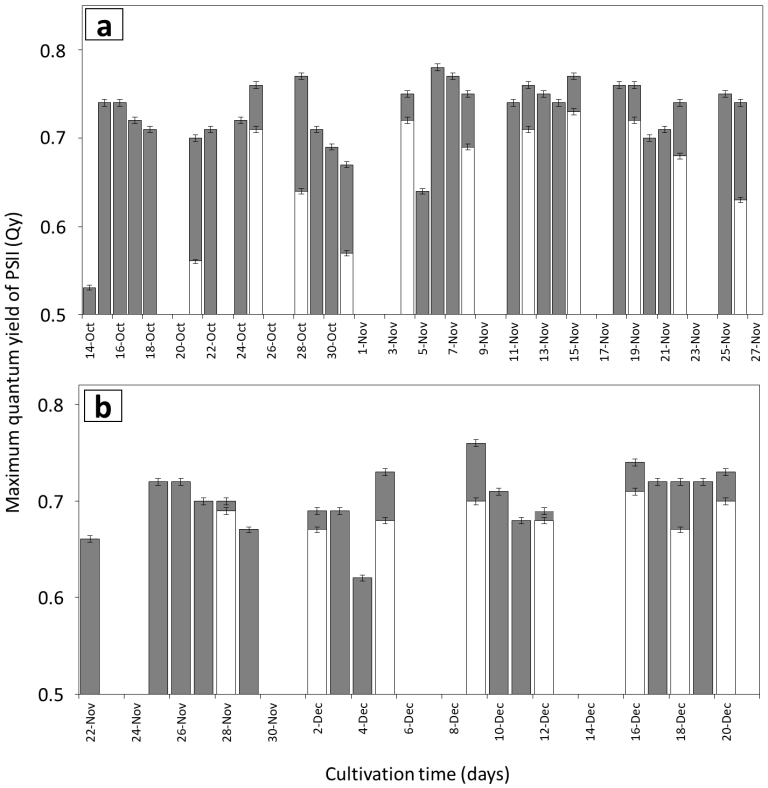

Figure A4. Maximum photosynthetic efficiency of photosystem II (PSII)—quantum productivity (Q_y) of the *T. striata* cultures in the time-course of the semi-continuous cultivation (**a**) for the 8000 L raceway pond outdoor (RW76out); (**b**) for the 45,000 L raceway pond outdoors (RW300out). Legend: gray bars—daily quantum productivity; white bars—quantum productivity of the culture right after the harvesting; in some cases the white bar is superimposed over the gray bar as both data are taken the same day—before and after the harvest. There is no statistical difference ($p > 0.05$) between quantum productivities for the cultures in two ponds: (**a**) RW76out: photosynthetic efficiency range $0.53 < Q_y < 0.78$, mean 0.71, median 0.72; (**b**) RW300out: photosynthetic efficiency range $0.62 < Q_y < 0.76$, mean 0.70, median 0.70.

Table A2. Heavy metal contents (in ng/g_{DW}) of *T. striata* biomass harvested from 8000 L indoor RW80in and outdoor RW76out ponds. We can assume *T. striata* biomass is free from As, Hg and Pb as these heavy metals' contents were below the detection limit in all tested samples. Statistical analysis showed no significant difference between samples for the elemental composition of two harvests of the outdoor pond RW76out ($p > 0.05$).

Element	RW80in	RW76out	RW76out
Units: ng/g_{DW}	**10-Sep**	**22-Nov**	**26-Nov**
Co	0	589 ± 26	618 ± 26
Cd	0	54 ± 0.5	54 ± 0.9
Cr	165 ± 8	3584 ± 324	3632 ± 260
Ni	0	4616 ± 409	4178 ± 263
Se	0	4627 ± 546	4971 ± 227
Al	0	49 ± 1.5	49 ± 0.6
Si	0	25 ± 2.1	26 ± 4.0
Mo	402 ± 20	0	0
As, Hg, Pb	0	0	0

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