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Impact of seasons on industrial cultivation of *Limnospira platensis* (Spirulina): A year-round case study on biomass, phycocyanin, and protein productivity in Portugal

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ABSTRACT

Spirulina is the most commercially produced biomass, among microalgae and cyanobacteria, with extensive applications across food, feed, nutraceutical and biotechnological sectors. While Spirulina cultivation is well-characterized at laboratorial and small-scale production, critical knowledge gaps remain regarding industrial-scale production in temperate climates. Specifically, there is a lack of robust empirical data on how seasonal environmental fluctuations affect year-round productivity and the maintenance of consistent biomass quality (protein, phycocyanin content) under large-scale operational constraints. This study evaluates the year-round industrial production of *Limnospira platensis* in large-scale (1000 m^2 and 4000 m^2) raceway reactors at Allmicroalgae - Natural Products S.A., Portugal. Biomass productivity as well as protein and phycocyanin contents across different seasons and throughout the day were assessed. The influence of environmental factors such as temperature and solar radiation on productivity and biomass composition was also analyzed. Industrially grown Spirulina reached an average annual productivity of 5.1–5.6 $g.m^{-2}.d^{-1}$. Seasonal variation revealed a winter productivity decline higher than 60 %, compared to peak yields of 7.5–7.6 $g.m^{-2}.d^{-1}$ from April to September. Protein content remained consistent year-round between 58.4 and 64.7 %, whereas phycocyanin content presented strong solar radiation dependence, peaking at 15.2 % in summer and decreasing to 10.4 % in winter. Furthermore, an analysis throughout the day identified an optimal harvesting window between midday and sunset to maximize phycocyanin levels. These findings validate the industrial feasibility of year-round Spirulina cultivation in temperate climates and provide seasonal productivity forecasting and harvest timing optimization, towards protein consistency and phycocyanin yield optimization.

1. Introduction

The global microalgal and cyanobacterial production has increased substantially in recent years, reaching a production of 56,456 tons in 2019 and a market size value of 12.8 billion US dollars in 2024 [1,2]. This growth reflects their rising industrial relevance and diverse applications across various sectors. Among these, *Limnospira platensis* and *Limnospira maxima,* commonly known as Spirulina, stand out as the most commercially cultivated species. In Europe alone, production accounts for 150 tons of dry biomass annually, fulfilling nearly half of the market demand [3,4]. Spirulina's high value is driven by its exceptional nutritional profile, particularly high protein content, typically between 50 and 60 %, making it a valuable dietary supplement and a key ingredient in the food and animal feed industries [5–7]. Additionally, Spirulina contains high concentrations of phycocyanin, a blue pigment-protein complex that constitutes up to 20 % of its dry weight. Phycocyanin is renowned for its antioxidant, anti-inflammatory, and anticarcinogenic properties, which promotes its increasing use in the nutraceutical and cosmetic and natural food colorant market [8–12]. The economic incentive for phycocyanin extraction is significant, as high-purity

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phycocyanin can drive prices up to USD 500 per kg, whereas raw Spirulina biomass is typically sold for less than USD 10 per kg [13]. Consequently, Spirulina is often cultivated specifically for phycocyanin extraction.

Large-scale microalgae production is conducted in photobioreactors that are classified into open systems, such as raceway (RW) ponds, or closed systems, such as tubular reactors [14]. Among these, RW ponds are the dominant reactors for Spirulina production. Consisting of shallow open tanks with recirculation driven by a paddlewheel, their widespread adoption is due to their operational simplicity and overall low capital and operational costs, compared to closed reactors [15]. However, this open design presents two primary challenges: susceptibility to contamination and inherent light limitation. The risk of contamination, whether biological or chemical, like dust or heavy metals, can be mitigated by cultivating extremophilic species like Spirulina, which thrives in high-alkalinity media, and/or by enclosing the ponds within a greenhouse [31]. The challenge of light limitation, caused by a low surface-to-volume ratio, necessitates operation at shallow depths (typically 10 to 20 cm) [12]. This operational constrain limits biomass concentrations to a maximum of 0.5–1.0 $g.L^{-1}$, with these systems achieving average productivities of approximately 10 $g.m^{-2}.d^{-1}$ [16,17].

Outdoor production in RW ponds is fundamentally affected by environmental factors. Solar radiation is the primary determinant of photosynthetic performance, while temperature is crucial for thermophilic species, such as Spirulina, with optimal growth between 30 and 35 °C [18,19]. Light exposure in open ponds is characterized by two crucial cycles: on a short timescale, turbulent mixing within the pond creates a “flashing light” effect, as cells circulate between the high-irradiance surface and the photo-limited dark zones at the bottom [20]; on a longer timescale, the culture is subject to the diurnal cycle, and variations in daily solar irradiance, which have previously have been reported to impact Spirulina's biochemical profile [21,22]. These radiation fluctuations trigger unique physiological modifications in cells as they adapt to the changing environmental conditions [20].

The feasibility of pilot and industrial-scale Spirulina cultivation in open RW ponds has been demonstrated across various subtropical and temperate regions, such as Italy [6,7], Spain [23–25], China [26], South Africa [27], South Korea [28], and Saudi Arabia [29] with reactors up to 660 m^2. Despite this progress, knowledge gaps persist regarding large-scale cultivation under temperate conditions. Specifically, there is a lack of robust empirical data on how seasonal environmental fluctuations affect year-round productivity and the maintenance of consistent biomass quality (protein, phycocyanin content, microbiological safety) under large-scale operational constraints.

Therefore, this study reports the annual production of Spirulina in industrial RW reactors at Allmicroalgae - Natural Products S. A. facilities, in Pataias, Portugal. This research focused on the biomass, total protein, and phycocyanin production throughout the year and in the two different scale reactors (1000 m^2 and 4000 m^2). To the authors' knowledge this is the first study reporting long-term cultivation of *L. platensis* in reactors with volumes between 1000 and 4000 m^3. Additionally, daily fluctuations of phycocyanin and protein content, as well as their dependence on temperature and solar radiation, are also studied at this industrial scale. This research provides valuable insights into large-scale cultivation of Spirulina, addressing key challenges in optimizing industrial biomass and bioactive compound production.

2. Materials and methods

2.1. Microalgae strain and culture medium

Limnospira platensis was obtained from Allmicroalgae's culture collection. Biomass production, from pilot to industrial scale, was conducted using an organic culture medium reported by Machado et al. [30]. The medium was supplied daily by a controlled dosing system to prevent nutrient limitation and maintain the nitrogen concentration under 1 mM.

2.2. Cultivation systems and operation

Industrial-scale Spirulina cultivation was carried out at Allmicroalgae - Natural Products S. A. facilities in Pataias, Portugal (coordinates: 39°39′15.5”N 8°59′23.6”W), from November 1st, 2023 to October 31st, 2024. Spirulina was grown in industrial RW ponds made from high density polyethylene sheets with areas of 1000 m^2 and 4000 m^2, as shown in Fig. 1, housed within a transparent plastic greenhouse (Indasol Tritermic from Solplast, Spain). Paddlewheels made from 316 L stainless steel (one per 1000 m^2 pond and two per 4000 m^2 pond) provided continuous culture agitation at an average speed of 0.16 $m.s^{-1}$. Culture depth ranged from 15 to 20 cm, depending on the seasonal evaporation rates. Fresh water was replenished daily to compensate for evaporation losses.

The cultures were monitored daily, with samples collected Monday to Friday at 8:30 AM, adjacently to the paddlewheel, where agitation is most efficient. The cultures were monitored by microscopic observation to detect biological contaminants using an Axio Scope A1 microscope (Carl Zeiss Microscopy GmbH, Oberkochen, Germany). The pH was measured daily (VWR, Amadora, Portugal), and the optical density was determined using a Genesys 10S UV–Vis spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA). Nutrient availability was tracked by determining the ammonium concentration with a commercial test kit (Ammonium-Ammonia Sera test, Sera, Germany) following the manufacturer's instructions. Culture quality was assessed weekly through the performance of the total viable colonies (TVC) analysis at 30 °C.

Dry weight (DW) was estimated through a previously established correlation with optical density at 600 nm [31,32], using Eq. 1 with a Pearson correlation coefficient of 0.9951. The temperature was recorded daily at 2:00 PM, and an internal ventilation system was activated when needed to maintain the culture's temperature under 35 °C.

$$DW\left(g.L^{-1}\right) = 1.75 \times OD_{600nm} + 0.0026 \left(n = 38, R^2 = 0.9902\right) \quad (1)$$

All raceway cultures were initiated from the same inoculum and had been operational since summer 2023, although data preceding the study period were not reported. Harvesting frequency and dilution rates were adjusted to maintain the biomass concentration between 0.6 and 1.0 $g.L^{-1}$. A volume between 30 and 60 m^3 was typically harvested per cycle, with the harvesting frequency adjusted to sustain target biomass concentrations. The 1000 m^2 raceways were harvested, on average, every three days during summer, twice a week during spring and autumn and once a month during winter. The 4000 m^2 raceway was harvested, on average, every day during summer, every other day during spring and autumn and once a week during winter. This translates to an average monthly harvest of 10 % and 50 % of the total volume of the 1000 m^2 and 4000 m^2 reactors, respectively, during winter. For the remaining seasons, the average turnover volumes were similar for the two reactor types: 175 % for spring and autumn and 200 % summer.

To save water and nutrients, permeate from the sieving process was routinely recirculated into the raceway ponds. However, this practice was suspended for culture medium renovation when the buildup of organic matter or the TVC surpassed internally established thresholds. The specific data on the frequency of these events are not detailed in this study, but these renovations were infrequent, occurring only as needed, typically involving replacement of approximately 6 % of the total reactor volume with fresh medium.

2.3. Biomass downstream processing

Biomass was harvested using a 25 μm mesh in a vibrating sieve (MR60 S 8–8-KL-C from Vibrowest, Italy). The harvested biomass was washed with fresh-water and concentrated using a hydraulic press

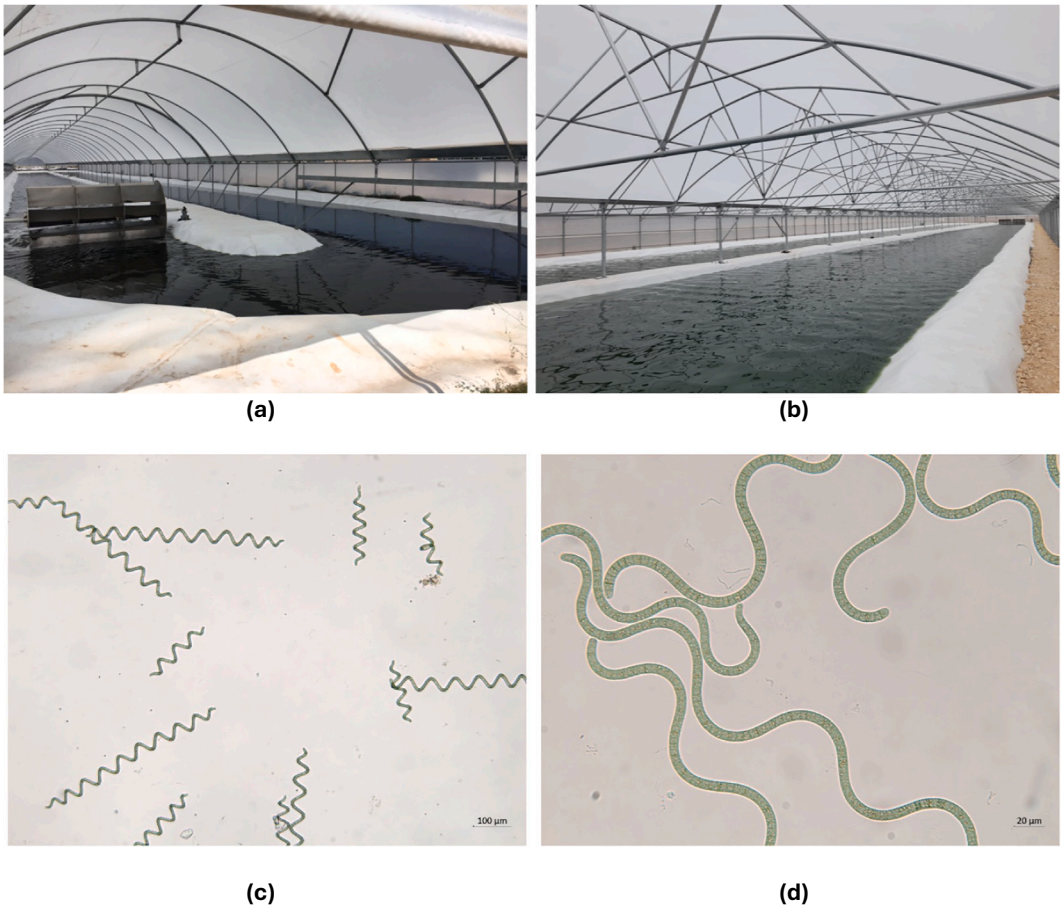

Fig. 1. – Raceway ponds of 1000 m^2 (a) and 4000 m^2 (b) used to cultivate Spirulina (*Limnospira platensis*) at Allmicroalgae - Natural Products S.A. (Pataias, Portugal) and microscopic observations of Spirulina grown in those reactors at a magnification of 100× (c) and 400× (d).

(KRWPR20F from Kraftwell, Russia) to a final biomass concentration of 150–200 $g.L^{-1}$. Biomass was then extruded into "spaghetti-like" filaments (2200 from VEVOR, China) and placed onto stainless steel mesh trays. This biomass was dried overnight using a hybrid solar drying system (Black Block – Hybrid Solar Drying Systems from Agroportal, Portugal), operating at a constant temperature of 40 °C, resulting in the final commercialized Spirulina "nibs". This hybrid drying system utilizes ambient air heated by solar radiation, when irradiance is sufficient. The system also uses grid electricity to power the ventilation fan for air circulation and to provide supplemental heat, via an auxiliary heater, when solar radiation is absent or insufficient.

2.4. Phycocyanin and protein contents analyses

The phycocyanin and total protein contents were analyzed for all the dried biomass batches during the production period. Additionally the daily variation of phycocyanin and protein content in the culture was also assessed in two 1000 m^2 RW ponds at different times of the day, during a 24-h period. For this, samples were collected at sunrise (7:00 and 8:00 AM), midday (1:00 and 2:00 PM), sunset (7:00 and 8:00 PM) and midnight (1:00 and 2:00 am) on three Spring/Summer days (24th and 27 of May and 7th of July 2022). The dry weight, phycocyanin and protein content of each sample, as well as of the dried biomass were analyzed as explained below.

Phycocyanin was quantified by the method developed by Bennett and Bogorad [33], with some minor modifications. First, phycocyanin was extracted by dissolving 10 mg of nibs (or an equivalent DW of previously frozen wet biomass) in 6 mL of 10 mM sodium phosphate buffer, pH = 7.0. The solution was vortexed for 5 min, with 0.5 mL of zirconia beads, followed by centrifugation (Hermle Z300 centrifuge, Gosheim, Germany) for 10 min at 2000 ×*g*. The supernatant was collected, and the extraction process was repeated with the remaining pellet until the supernatant was colorless. Phycocyanin concentration ([PC]) was measured spectrophotometrically using Eq. 2, where A_{620nm}, A_{652nm} correspond to the absorbance at 620 and 652 nm, respectively [33]. Biomass' phycocyanin content (PC) was finally calculated by Eq. 3,

$$[PC]\ \left(mg.L^{-1}\right) = \frac{A_{620nm} - 0.474 \times A_{652nm}}{5.34} \tag{2}$$

$$PC\ (\%, w/w) = \frac{[PC] \times V_{solvent}}{m_{biomass}} \tag{3}$$

where the [PC] was multiplied by the volume of added solvent ($V_{solvent}$) and divided by the added biomass dry weight ($m_{biomass}$).

The total protein content of nibs or freeze-dried culture was determined by multiplying the percentage of N by the 6.25 [34]. The total N content of dried biomass was analyzed by an external certified laboratory where it was determined by the Dumas method in a nitrogen and protein analyzer (FP828p, Leco, USA).

Seasonal average phycocyanin and protein contents were calculated by the average content of all the analyzed nibs' batches of that season.

2.5. Dried Spirulina microbiological and heavy metal analyses

Microbiological and heavy metal analysis were performed by an accredited external laboratory using the solar dried biomass. Microbiological assessments included TVC at 30 °C and screening for regulated pathogens, including *Staphylococcus aureus*, *Listeria monocytogenes*,

Escherichia coli and *Salmonella* sp. Heavy metal analysis quantified lead, cadmium, mercury, arsenic, and tin concentrations. While specific data are not presented in this report, all the batches were confirmed to remain below the legal limits for all tested parameters throughout the study.

2.6. Determination of biomass, phycocyanin and protein productivities

The biomass productivity was determined monthly for each RW by the quotient of the harvested biomass ($m_{harvested}$, in dry weight) plus the biomass difference in the RW during that month ($\Delta m_{raceway}$), by the average RW volume ($V_{average}$) and number of days of that month (t) (Eq. 4). Eq. 5 represents the calculation of $\Delta m_{raceway}$ in which X represents the biomass concentration in the RW and V the RW volume at the end (f) and beginning (i) of the month.

$$P_{biomass}\ (g.m^{-2}.day^{-1}) = \frac{m_{harvested} + \Delta m_{raceway}}{V_{average} \times t} \quad (4)$$

$$\Delta m_{raceway}\ (g) = X_f \times V_f - X_i \times V_i \quad (5)$$

Phycocyanin and protein productivities were determined seasonally as the average of the protein or phycocyanin productivity of each batch produced in that season. The seasonal batch productivity was determined by multiplying the respective batch's phycocyanin or protein content by the biomass productivity of the corresponding harvest month.

2.7. Determination of photosynthetic efficiency

The monthly photosynthetic efficiency (PE) was determined by multiplying the average Spirulina's higher heating value (HHV) by the ratio of the average areal productivity $P_{biomass}$ divided by 90 % (radiation that the greenhouse used in this study transmits) of the average total incident solar radiation of that month (Eq. 6). The specific HHV ($kJ.g^{-1}$) was calculated according to a previous correlation reported by Callejón-Ferre et al. [35], presented in Eq. 7, where C represents the percentage of carbon, H the percentage of hydrogen and N the percentage of nitrogen obtained by the CHN analysis of the final biomass.

$$PE\ (\%) = HHV \times \frac{P_{biomass}}{0.9\ Incident\ radiation} \times 100 \quad (6)$$

$$HHV\ (kJ.g^{-1}) = -3.393 + 0.507C - 0.341H + 0.067N \quad (7)$$

The solar radiation and temperature were measured using a 7-in-1 weather station (Bresser, Germany) in Pataias, Portugal. The average monthly incident radiation was calculated by averaging the daily incident solar radiation. The average, maximum and minimum monthly temperature were obtained by averaging the daily, average, maximum and minimum temperature of that month.

2.8. Statistical analysis

Statistical analyses were performed using R software (version 4.1.2) through the RStudio IDE (version 2023.06.1). Results are presented with a 95 % confidence level. The normal distribution of data was tested through the Shapiro–Wilk test and the data homogeneity was tested using the Bartlett test. The data was then compared using one-way ANOVA and Tukey's multiple comparison tests. The non-homogeneous data was compared with the Kruskal–Wallis test, followed by Dunn's test with a Bonferroni p-value adjustment.

3. Results and discussion

3.1. Environmental conditions

The average monthly ambient temperature varied between 11.5 and 19.0 °C, in December and August, respectively (Fig. 2, a). The smallest thermal amplitude was registered in February, which presented a 6.4 °C difference between the average minimum and maximum temperatures, while April presented the highest amplitude of 9.7 °C. Thus, this study was conducted in a temperate climate, with mild winters (average temperatures of 12.4 °C) and summers (average 18.8 °C). In contrast, daily incident solar radiation fluctuated significantly between 4.7 and 24.1 $MJ.m^{-2}.d^{-1}$ in December and May, respectively (Fig. 2, b). The cultures' temperature varied between 16.0 °C in December and 30.6 °C in July, a direct consequence of both ambient temperature and solar radiation. Notably, the maximum temperature was controlled by a ventilation system that was activated to maintain the culture's temperature below 35 °C in warmer months.

The significant temperature differential between the average ambient and the culture temperature, reaching 12 °C during summer, underscores the greenhouse's heat-trapping efficiency. Such systems are beneficial in temperate regions, like the present study, since Spirulina's optimal temperatures are reported to be between 30 and 35 °C, temperatures much higher than the registered average ambient temperatures (< 20 °C). However, the greenhouse poses a risk of thermal stress on the hottest days. Therefore, the existing ventilation system is crucial for temperature management. To further mitigate this risk and prevent potential yield losses, the addition of advanced climate control systems, such as automated shading or evaporative cooling, could be useful for a more precise control of peak temperatures.

3.2. Year-round biomass productivity and cultivation efficiency

The monthly average biomass productivity (Fig. 2, c) ranged from 1.1 to 8.2 $g.m^{-2}.day^{-1}$ in the 1000 m^2 RW and from 1.1 to 8.8 $g.m^{-2}.day^{-1}$ in the 4000 m^2, corresponding to an annual average of 5.1 and 5.6 $g.m^{-2}.day^{-1}$, respectively, for the different size reactors. Winter presented the lowest productivity with 1.7 and 2.8 $g.m^{-2}.day^{-1}$, while summer recorded the highest values with 7.3 and 7.6 $g.m^{-2}.day^{-1}$ for the 1000 m^2 and 4000 m^2 RW, respectively. This seasonal drop in productivity is consistent with previous studies of Spirulina grown in outdoor RWs. Jimenez et al. [23,24] reported winter Spirulina productivities to be around 2 $g.m^{-2}.day^{-1}$, and Kurpan et al. [6] observed a 2.5-fold decrease in winter productivity, compared to summer, both observations aligned with the findings of the present study. The drop in winter productivity observed in the present study might be attributed to the lower availability of solar radiation (4.7–9.0 $MJ.m^{-2}$), and by the suboptimal culture temperatures of December and January (16–17 °C). Spirulina growth is reportedly negligible below 17 °C [6], which likely explains the low productivity during this period. To mitigate this thermal limitation and increase winter productivity, one strategy would be to install a heating system [36]. An example would be a solar collector that would capture solar energy during the day, store it in an insulated water tank, and then circulate the heat through the pond during the colder night-time hours to maintain a more favorable temperature for growth. The peak productivities registered during summer, with culture temperatures reaching 28.3 to 30.6 °C, directly reflect the culture operating near its optimal thermal range of 30–35 °C [37] [38]. This confirms, that in a temperate climate, summer provides the ideal conditions for maximizing Spirulina growth. These results are in accordance with the growth model proposed by Rodríguez-Miranda et al. [39] which predicted summer as the most favorable season for Spirulina cultivation in Spain. Moreover, long-term industrial cultivation studies, in similar latitudes like Spain and Italy, also presented an increase in productivities during Summer [24] [40].

The highest recorded productivity was 8.8 $g.m^{-2}.day^{-1}$, exceeding the 7.6 $g.m^{-2}.day^{-1}$ in a 55 m^2 RW reported by Pistocchi et al. [7] in northern Italy and the 6.0 $g.m^{-2}.day^{-1}$ reported by Guidi et al. [41] in an 8 m^3 RW in the Canary Islands. However, this productivity was lower than the 16, 12.2 and 11.1 $g.m^{-2}.d^{-1}$ obtained in RWs of 300 m^2 in northern Italy [6], 450 m^2 in southern Spain [24] and 660 m^2 in Inner

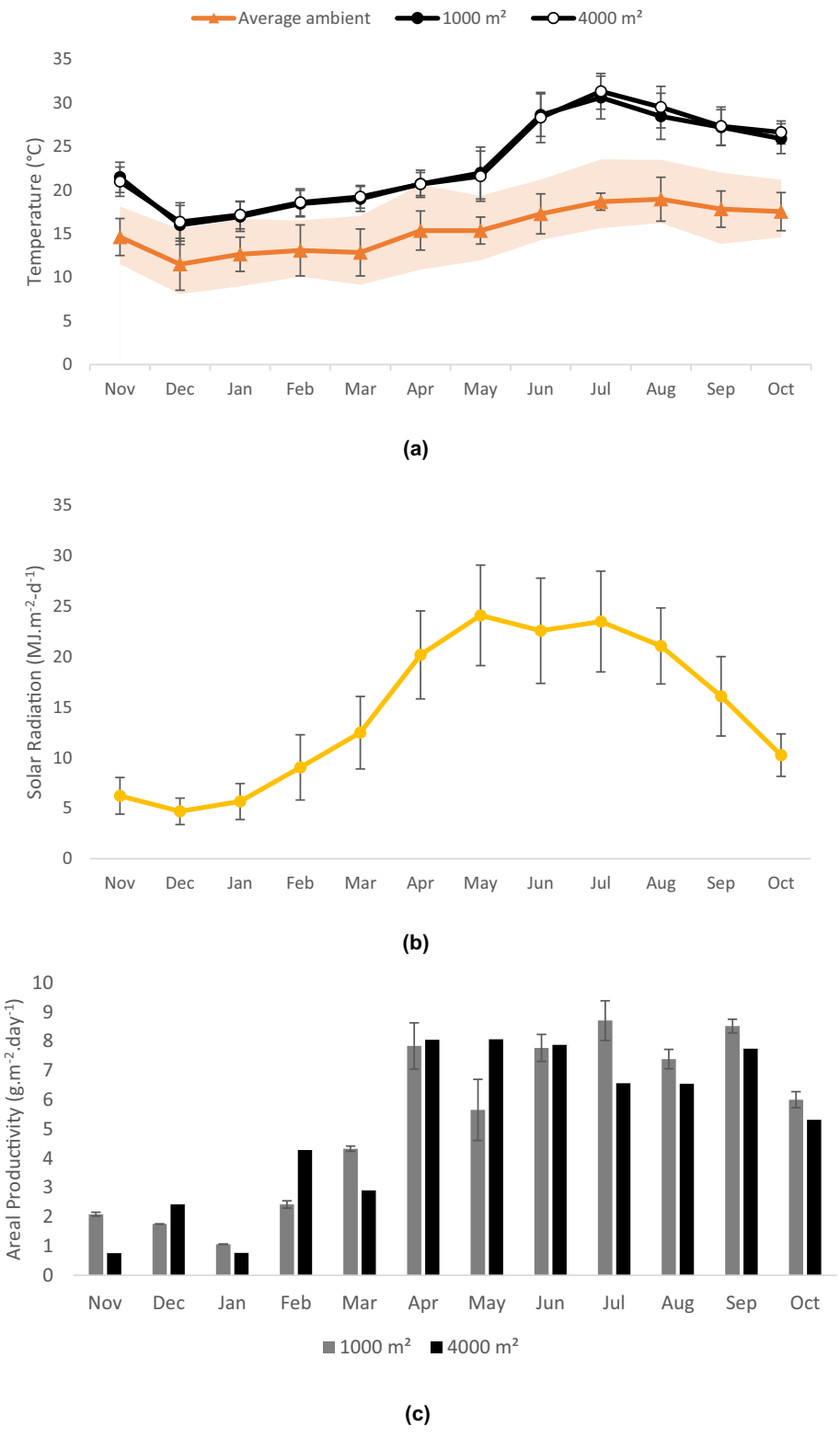

Fig. 2. – Monthly average environmental conditions and biomass productivity for Spirulina cultivation in Pataias, Portugal from November 1st, 2023 and October 31st, 2024. (a) Monthly average ambient temperature (solid line) with maximum and minimum range (shaded area), and culture temperature of both raceways; (b) Average daily incident solar radiation; (c) Monthly average areal biomass productivity in 1000 m^2 and 4000 m^2 raceway ponds.

Mongolia (China) [22], respectively. This variation suggests that biomass productivity depends not only on the RW location and respective available temperature and solar radiation, but also on its size and the operation systems used.

Excluding winter months, the annual productivity increased to 6.5 and 6.0 $g.m^{-2}.d^{-1}$, while the most productive half of the year (April to September) reached an average productivity of 7.6 and 7.5 $g.m^{-2}.day^{-1}$, for the 1000 m^2 and 4000 m^2 RW, respectively. This period coincides with higher culture temperature (21–31 °C) and solar radiation levels (16–24 $MJ.m^{-2}.d^{-1}$), both favorable for Spirulina growth. Short

production seasons of 6–7 months, namely from April to October, had already been reported by other industrial companies from latitudes higher than the equator, due to low temperatures in the remaining year [42]. The values of the present study align with those reported in China (5.3–9.1 $g.m^{-2}.day^{-1}$), by Lu et al. [26] under similar annual radiation (5730–5930 $MJ.m^{-2}$), compared to that of the present study (5358 $MJ.m^{-2}$). Interestingly, these values are also comparable to the annual average productivity reported by Jimenez et al. [24] in southern Spain (8.2 $g.m^{-2}.day^{-1}$).

Ultimately, the results of the present study align with values reported from other successful European cultivation sites, as shown in the comparative summary in Table A1. Furthermore, they establish a clear baseline for year-round biomass productivity in a temperate industrial setting, demonstrating competitive yields, not only during the peak summer productivities, but also in an extended production season from late Spring to early Autumn.

Fig. 3 illustrates the monthly PE of the culture, ranging from 0.3 to 1.1 %. These values remained relatively stable throughout the year and across the different reactors, indicating that biomass productivity variations were primarily driven by seasonal fluctuations in solar radiation, rather than changes in the culture's intrinsic photosynthetic performance. This suggests that addressing the severe light limitation is crucial for improving winter productivity. One approach is to install supplemental lighting, such as submersible LED systems [43]. These can serve two key functions: extending the daily photoperiod into the early morning and evening, and boosting light intensity on overcast days to ensure the culture receives a more consistent daily light dose. A complementary operational strategy is to adjust the culture's physical parameters, for instance reducing the culture depth while maintaining a lower biomass concentration would increase the average light exposure per cell, thereby making more efficient use of the limited winter sunlight.

The PE values from the present study are slightly below the 1.5 % reported by Soni et al. [44] for Spirulina grown in RWs. Nevertheless, the values are comparable to those obtained by Cunha et al. [45] of 0.53–0.65 % for *Nannochloropsis oceanica,* grown from February to July in RWs at the same location (Pataias, Portugal).

3.3. Energy consumption for culture agitation

The energy required for Spirulina cultivation was quantified by measuring the energy expenditure by the paddle wheels of each raceway (1000 and 4000 m^2), whose velocity was maintained throughout the year, regardless of biomass growth. In Fig. 4, the average energy demand per kilogram of biomass in each month is displayed. Since the RW agitation velocity remained constant, energy costs per kg of biomass were inversely proportional to productivity. From March to October, energy expenditure ranged from 2 to 9 $kJ.kg^{-1}$, increasing significantly during the low productivity months (November–February), reaching up to 37 $kJ.kg^{-1}$. These values are considerably lower than the energy consumption reported for the RW cultivation of *Chlorella* sp. by Collet et al. [46] and Zeng et al. [47], of 720 and 7900 $kJ.kg^{-1}$, respectively. Interestingly, despite the 4000 m^2 RW being powered by two paddlewheels, it consistently exhibited lower energy costs per kilogram of biomass, compared to the 1000 m^2 RW, while maintaining similar areal productivities. This highlights the efficiency gains achieved through a 4-fold scale-up, demonstrating the benefits that larger cultivation systems have in reducing energy consumption per unit of biomass produced. It is important to note that these calculations reflect only agitation energy inputs. Additional energy demands, such as those related to the ventilation cooling system (most significant from June to August) and downstream processing (grid-dependent in winter during reduced solar radiation) were not accounted for. While these factors would affect seasonal energy distribution patterns, they would have minimal impact on the comparative efficiency analysis between the two raceway scales.

3.4. Seasonal variation in protein and phycocyanin content of dried Spirulina

Seasonal variations in phycocyanin (Fig. 5) and total protein content and productivities (Fig. 6) were analyzed in the solar dried Spirulina. Phycocyanin content was significantly lower ($p < 0.05$) during winter (10.4 %), compared to the other seasons. The highest recorded phycocyanin values of 15.2 % corresponded to biomass produced in the summer but did not differ significantly from those of spring (15.1 %) or autumn (13.5 %). Phycocyanin productivity mirrored this trend, being over three times lower in winter than in any other season. This reduction is in accordance with the findings of García-López et al. [48] and de Jesus et al. [49], who reported lower phycocyanin contents in biomass cultivated under lower temperatures and reduced solar radiation. Despite these seasonal variations, the phycocyanin content observed in this study (10–15 %) remains within the range of 5–20 % reported in the literature [7,22,23,48] confirming the production of high-quality biomass throughout the seasons. Moreover, the minimum phycocyanin content of this study (10 %) is higher than the yearly average 6 % reported by Jimenez et al. [24], the autumn values of 8–10 % reported by García-López et al. [48] and the summer phycocyanin content of 8–10 % reported by Yang et al. [22].

Protein productivity presented a similar trend to the phycocyanin productivity, without statistically significant seasonal variations

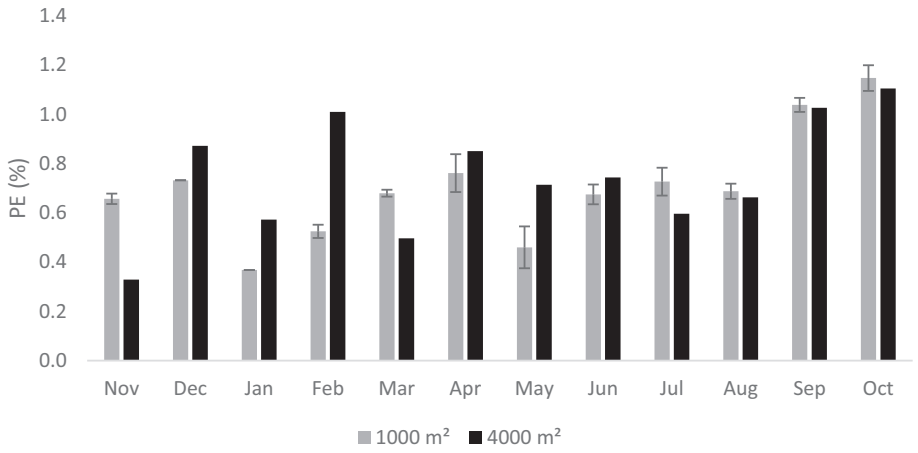

Fig. 3. – Monthly photosynthetic efficiency (PE) of Spirulina cultivated in two 1000 m^2 and one 4000 m^2 raceway ponds. Spirulina was cultivated from November 1st, 2023 until October 31st, 2024 in Pataias, Portugal. The error bars in the 1000 m^2 represent the maximum and minimum productivity values obtained in that month between two replicate ponds.

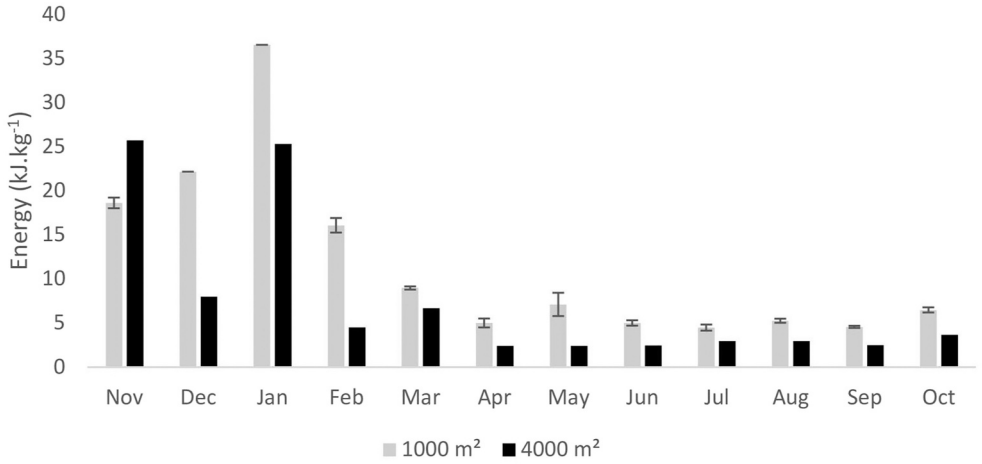

Fig. 4. – Monthly average energy consumption for culture mixing per kilogram of Spirulina biomass produced in 1000 m^2 and 4000 m^2 raceway ponds from November 1st, 2023 until October 31st, 2024. Values for the 1000 m^2 ponds represent the average of two reactors, with error bars indicating the maximum and minimum values, while data for the 4000 m^2 pond represents a single reactor.

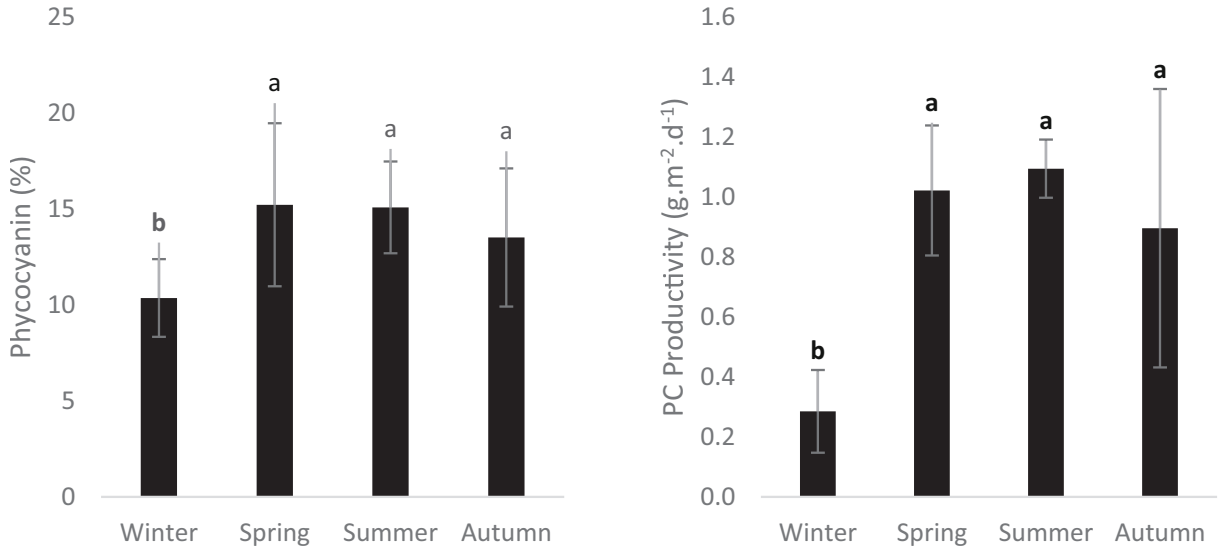

Fig. 5. – Seasonal phycocyanin content (%) and phycocyanin (PC) productivity ($g.m^{-2}.d^{-1}$) of solar-dried Spirulina grown in 1000 m^2 and 4000 m^2 raceway ponds. Nibs were produced from November 1st, 2023 until October 31st, 2024 in Pataias, Portugal. The values and respective error bars represent the average and standard deviation of the phycocyanin content and productivity of all the dried batches in each season. Different letters indicate statistically different values ($p < 0.05$).

between summer, spring and autumn, remaining within 4.0 to 4.4 g. $m^{-2}.d^{-1}$, meanwhile presenting a 50 % decrease in winter. On the contrary, protein content remained relatively stable throughout the year, with values ranging between 58.4 % and 61.6 % in winter, spring, and summer, and slightly (though statistically not significant), higher content in autumn (64.7 %). These values are within the range described in the literature for outdoor Spirulina production (50–70 %) [6,48,50], demonstrating that high-protein Spirulina can be industrially produced year-round, at the study's location. This divergence between stable total protein and variable phycocyanin content can be justified as a cellular resource allocation strategy under the low-light and low-temperature stress of winter. Literature studies reported that while the total protein content of Spirulina can remain stable under environmental stress, specific proteins are selectively adjusted to maintain cell viability. Under energy-limiting conditions, the cell downregulates the synthesis of its metabolically expensive, light-harvesting phycobilisomes (containing phycocyanin) to conserve resources. Furthermore, these pigments can be catabolized to remobilize nitrogen for more critical metabolic functions necessary for survival [51] [52].

The stability of protein content observed in our study aligns with some literature, although findings on this topic are inconsistent. For instance, Kurpan et al. [6] observed a 20 % decrease in Spirulina's protein content during winter, and García-López et al. [48] found a 15 % drop in protein content in Spirulina produced in autumn, compared to spring/summer. Similarly to the present study, Pistocchi et al. [7], did not find variations in the protein content of Spirulina when comparing different months or seasons. It is possible that the protein fluctuations observed in the present study may be influenced by nutrient concentration in the culture, dilution rates and pH variations, despite the efforts to keep these parameters constant. These assumptions are supported by Villaró et al. [50] and Andrade et al. [53] who reported that nutrient limited Spirulina cultures will present lower protein levels. Additionally, Yang et al. [22] suggested the protein content varies with the culture growth phase or adaptation to new conditions, while Mehar et al. [54] pointed pH as a factor that affects protein content.

The phycocyanin and protein contents and productivities in the 1000 or 4000 m^2 RWs were compared (Table 1) in the most productive half of the year (April to October 2024). The 4000 m^2 RW consistently

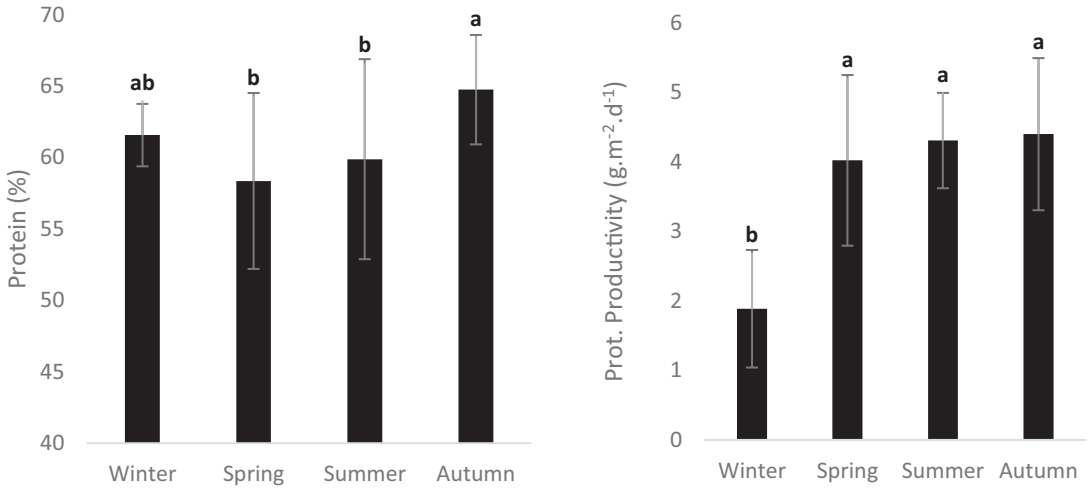

Fig. 6. – Seasonal protein content (%) and protein productivity ($g.m^{-2}.d^{-1}$) of solar-dried Spirulina grown in 1000 m^2 and 4000 m^2 raceway ponds. Nibs were produced from November 1st, 2023 until October 31st, 2024 in Pataias, Portugal. The values and respective error bars represent the average and standard deviation of the protein content and productivity obtained in each batch of that season. Different letters indicate statistically different values ($p < 0.05$).

Table 1
– Phycocyanin and protein content of solar dried Spirulina produced in two 1000 m^2 and one 4000 m^2 raceway ponds from April 1st to October 31st 2024 and areal productivity of those compounds during the same period. Values are presented as average and standard deviation of each batch produced during that period, by the respective reactors. Different letters indicate statistically different values ($p < 0.05$).

Analyzed parameter	1000 m^2 Reactor	4000 m^2 Reactor
Phycocyanin content (%)	13.7 ± 2.8^b	16.4 ± 2.9^a
Phycocyanin Productivity ($g.m^{-2}.d^{-1}$)	0.9 ± 0.1^b	1.2 ± 0.3^a
Protein content (%)	55.3 ± 6.2^b	63.7 ± 4.9^a
Protein productivity ($g.m^{-2}.d^{-1}$)	4.0 ± 0.6^b	4.6 ± 0.7^a

exhibited significantly higher values for all these parameters, suggesting that scale-up might enhance both biomass quality and overall productivity of phycocyanin and total protein. Since both systems operated under identical conditions, the only difference being an additional paddlewheel in the larger RW, it is likely that improved culture agitation contributed to greater radiation exposure and aeration, thereby enhancing protein and phycocyanin synthesis. This suggests that hydrodynamic optimization is a critical parameter for success. For future large-scale reactor designs, employing Computational Fluid Dynamics modeling could optimize reactor geometry and mixing systems, ensuring homogeneous light and nutrient distribution and eliminating dead zones.

This study confirms that high-quality biomass with stable protein content can be produced year-round, while phycocyanin content is

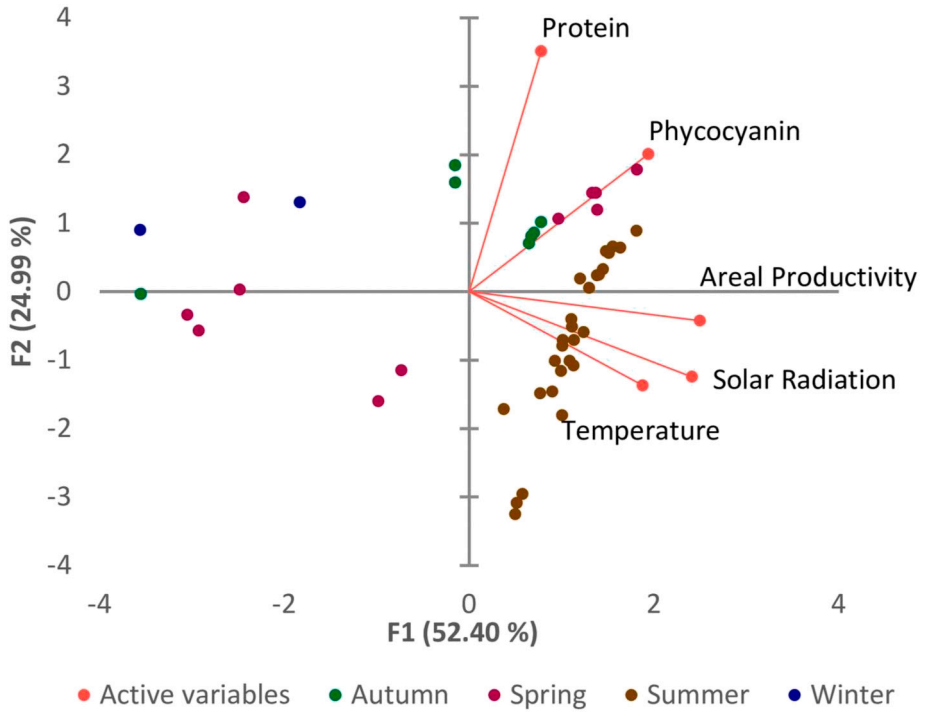

Fig. 7. – Principal Component Analysis (PCA) biplot showing the correlation between environmental factors (solar radiation, temperature) and production parameters (Spirulina areal productivity, phycocyanin content, and protein content). Data points are colored by season.

strongly influenced by seasonal conditions. As shown in Table A1, our results fit well within the range of productivity reported from large-scale operations in similar latitudes, while demonstrating a superior biochemical profile in terms of protein and phycocyanin content compared to several other studies.

3.5. Influence of abiotic parameters on biomass productivity and composition

Fig. 7 illustrates the Pearson linear correlation between Spirulina's areal productivity and key environmental factors, specifically culture temperature and incident solar radiation. Areal productivity presented a strong positive correlation with solar radiation ($R = 0.78$, $p < 0.05$) and a moderate correlation with temperature ($R = 0.57$, $p < 0.05$). Furthermore, areal productivity increased proportionally to the temperature, up to 35 °C (Fig. 8). A similar trend was observed for solar radiation, where increasing irradiance corresponded to higher productivity values, up to 20 $MJ.m^{-2}.d^{-1}$, after which productivities appeared to plateau (Fig. 8). These findings support the data presented in section 3.3 that indicated that productivity depends on both these factors. Similar trends were reported by Jiménez et al. [23] in 450 m^2 raceways, although their study did not present a saturation effect for high values of radiation. This discrepancy may be attributed to the lower radiation values reported in the abovementioned study, which did not surpass the 21 $MJ.m^{-2}.d^{-1}$.

Phycocyanin content also appears to be influenced by solar radiation, showing a moderate positive correlation, ($R = 0.57, p < 0.05$), with increasing radiation levels associated with enhanced phycocyanin content. In contrast, phycocyanin exhibited a non-significant correlation with temperature ($R = 0.14$, $p > 0.05$) (Fig. 9). Conversely, protein content did not exhibit a significant correlation with either of these abiotic factors ($R = 0.075$, $p > 0.05$ and $R = -0.125$, $p > 0.05$, for temperature and solar radiation, respectively), which is supported by Fig. 9.

This analysis statistically validates the observed seasonal trends, confirming that solar radiation is the primary driver for both biomass and phycocyanin productivity, while protein content remains remarkably robust against environmental fluctuations.

3.6. Daily variation of culture phycocyanin and protein contents

The biomass' daily variation of phycocyanin and protein content was assessed during the most productive seasons (spring/summer) (Fig. 10). Phycocyanin content increased with temperature and solar radiation, peaking at 15.2 % around the 02:00 PM, then decreasing to 12.5 % at 02:00 AM. In contrast, protein content showed no significant daily variation, ranging between 56 and 59 %. The stability of the total protein content, despite the significant fluctuation in phycocyanin, is not unexpected and can be explained by protein remodeling. This biological strategy involves cells degrading less essential proteins, such as those produced for different metabolic needs during the dark phase, to provide the amino acid building blocks for synthesizing phycocyanin [55]. Overall, these results indicate that to optimize biomass quality, harvesting between midday and sunset, and night-time harvesting should be avoided.

The observed peak in phycocyanin during maximum solar radiation can be attributed to three primary biological roles. Phycocyanin is the primary photosynthetic pigment, being involved in the channeling of solar energy [56]. For this reason, its synthesis is expected to increase when light is induced after a dark period maximizing energy capture from sunlight. Furthermore, phycocyanin is a powerful antioxidant, and its increased production serves as a protective mechanism against harmful reactive oxygen species that can be generated by high irradiance [56]. Under high light, Spirulina can fix carbon very quickly, and cells can try to balance this by storing nitrogen. This storage can be performed through the synthesis of phycocyanin which functions as a cellular nitrogen reservoir [57,58].

The observed phycocyanin variation is in accordance with the findings of Matallana-Surget et al. [59], who described a positive light-dependent increase in phycocyanin levels via direct measurements and proteomic analysis. However, these results appear to contradict those of Hidasi & Belay [21] who reported phycocyanin degradation with increasing temperature and radiation throughout the day [21]. A key difference between the previous report and the present study is that the RW was housed within a greenhouse, which reduces incident solar radiation by 20–50 % [21]. Phycocyanin is a light-sensitive pigment known to be degraded by high-intensity radiation, particularly UV light [60]. Although ambient radiation levels were similar, the actual irradiance received by the culture in the present study was lower, likely preventing photodegradation. Yang et al. [22] also reported a decline in phycocyanin levels with the increase of solar radiation during the day in an outdoor greenhouse-covered RW. In this case, the differences might be explained by the higher culture density of the present study of 100–130 $g.m^{-2}$, compared to 50–70 $g.m^{-2}$ reported by Yang et al. [22], which leads to a more significant light attenuation within the water column, reducing photoinhibition. Nonetheless, these authors also observed stable daily protein levels, consistent with the findings of the present study.

These daily variations in phycocyanin and protein content mirror their seasonal trends, further supporting the correlation between phycocyanin content and solar radiation while reinforcing the lack of correlation between protein content and abiotic factors. These findings

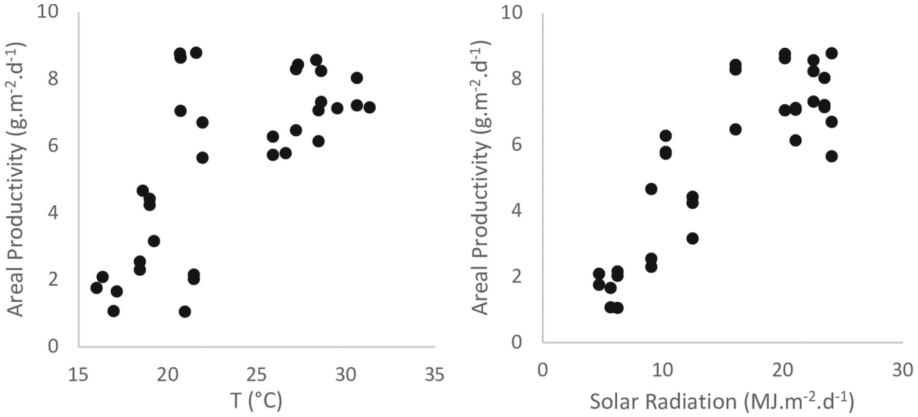

Fig. 8. – Correlation between the monthly areal productivity of Spirulina in three raceway ponds (two 1000 m^2 and one 4000 m^2) and their correspondent monthly average culture temperature and ambient solar radiation. The values correspond to the monthly averages collected from November 1st, 2023 until October 31st, 2024 in Pataias, Portugal.

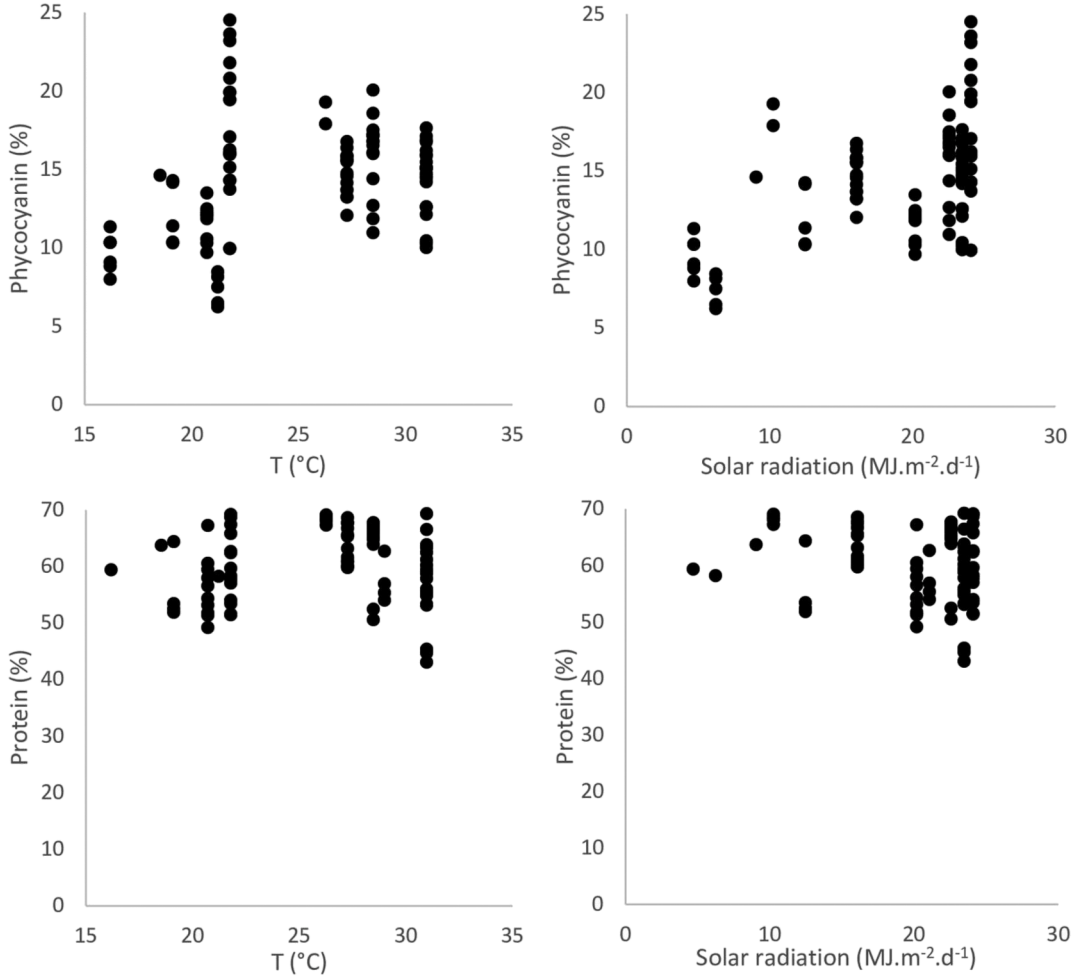

Fig. 9. – Correlation between the batch phycocyanin and protein content of Spirulina in three raceway ponds (two 1000 m^2 and one 4000 m^2) and their correspondent average culture temperature and ambient solar radiation. The values correspond to the monthly averages collected from November 1st, 2023 until October 31st, 2024 in Pataias, Portugal.

offer a powerful yet simple process optimization tool. By scheduling harvests between mid-day and late afternoon, when phycocyanin content peaks, producers can maximize the concentration of this high-value pigment in the final product. This strategy is particularly critical for enhancing biomass quality during winter, when baseline phycocyanin levels are at their lowest.

4. Conclusion

This study provides a comprehensive analysis of the annual production of L. *platensis* biomass, total protein, and phycocyanin in industrial-scale RW reactors (1000 m^2 and 4000 m^2) at Allmicroalgae - Natural Products S.A., in Portugal. The annual average productivity of 5.1–5.6 $g.m^{-2}.d^{-1}$ is in accordance with previous studies under similar climatic conditions, while peak productivity of 8.8 $g.m^{-2}.d^{-1}$ highlights the efficiency of large-scale systems, surpassing results from prior studies. Furthermore, scaling up from 1000 m^2 to 4000 m^2 RWs resulted in approximately 35 % reduction in energy consumption used for culture agitation per unit of produced biomass, highlighting the advantages of large-scale cultivation regarding energy efficiency and confirming a purported economy of scale. High-quality biomass was consistently produced, despite the seasonal fluctuations, with stable protein contents (58–65 %) and phycocyanin contents peaking in spring and summer (~15 %). A strong correlation between solar radiation and phycocyanin content was observed, whereas protein content remained unaffected by abiotic factors, such as temperature and radiation. Additionally, daily phycocyanin variations suggest midday to sunset as the optimal harvesting window to maximize biomass quality.

Overall, this study demonstrates the feasibility of semi-continuous large-scale Spirulina cultivation in temperate climates, ensuring a high protein and phycocyanin content throughout the year.

Glossary

RW raceway
TVC total viable count
PE photosynthetic efficiency

CRediT authorship contribution statement

Inês Guerra: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Cátia Torres:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Conceptualization. **Helena Cardoso:** Writing – review & editing, Validation, Supervision, Conceptualization. **Hugo Pereira:** Writing – review & editing, Validation, Supervision, Conceptualization. **Mafalda Trovão:** Writing – review & editing, Validation, Conceptualization. **Alexandre M.C. Rodrigues:** Writing – review & editing, Validation. **João Varela:** Writing – review & editing, Validation, Supervision, Conceptualization.

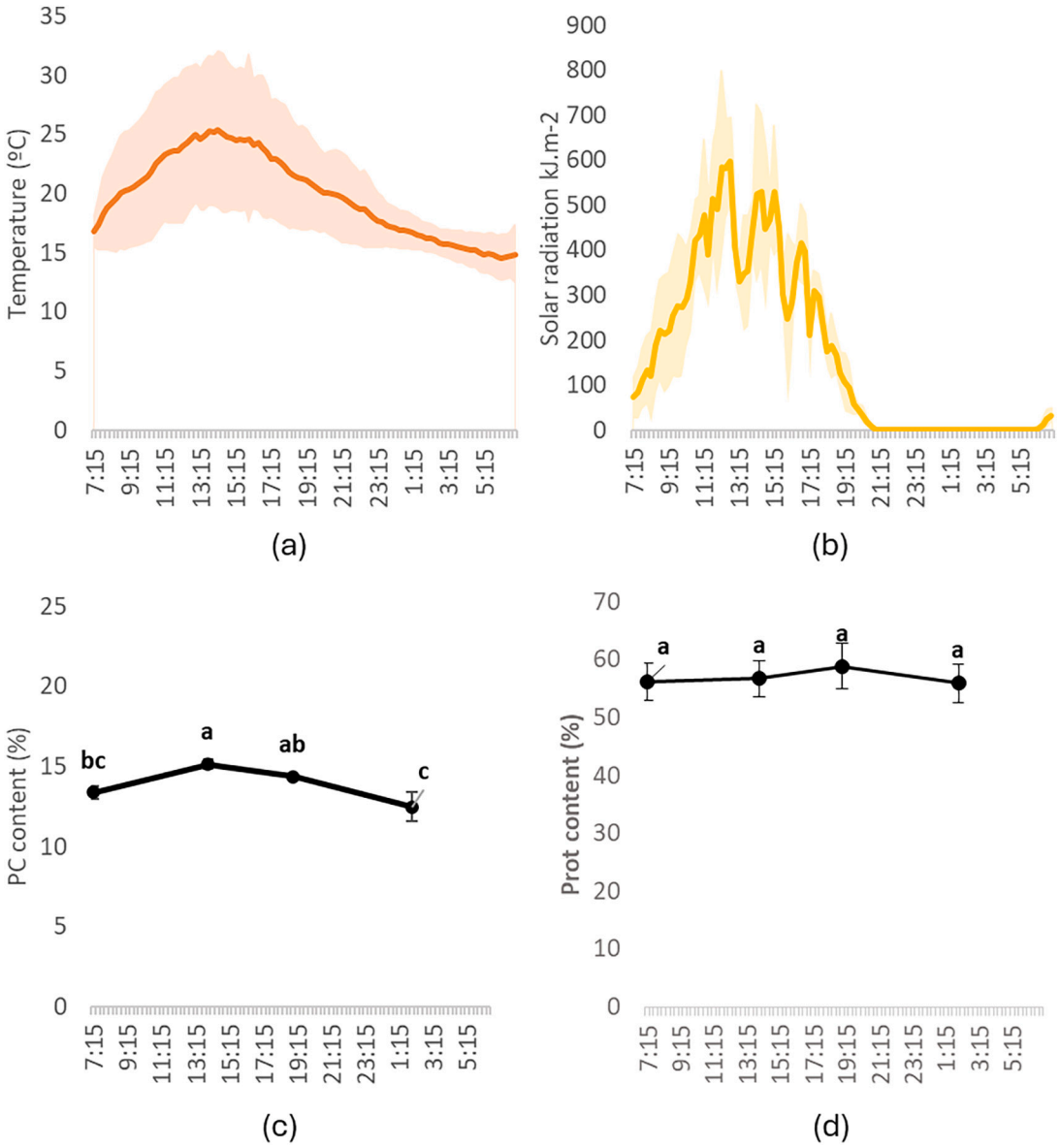

Fig. 10. – Daily (24-h) variation of ambient temperature (a), solar radiation (b), phycocyanin (PC) (c) and protein (d) contents (% of DW) of Spirulina biomass grown in 1000 m^2. Temperature and radiation data (a, b) show the average (solid line) ± standard deviation (shaded area) across three representative spring/summer days (May 24th, 27th and July 5th, 2022). Phycocyanin and protein content (c, d) are averages (± standard deviation) from two 1000 m^2 raceways over the same three days. Different letters indicate statistically significant differences between time points ($p < 0.05$).

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Declaration of competing interest

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

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Appendix A

Table A1
– Comparison between the present study and previous studies on pilot and industrial-scale Spirulina production.

Location	RW dimensions	Study Period	Biomass Productivity (g. $m^{-2}.d^{-1}$)	Phycocyanin Content (%)	Protein Content (%)	Reference
Pataias, Portugal	1000 m^2 and 4000 m^2	Year-round	7.5–7.6 (Apr–Sep) 5.1–5.6 (average)	10.4–15.2	58.4–64.7	Present study
Almería, Spain	80 m^2	summer	15.9–29.3	–	62.2	[50]
Málaga, Spain	450 m^2	September – July	10.3 (Mar–Nov) 8.2 (average)	6	47	[24]
Gran Canaria, Spain	80 m^2	September–November	6.0	6.0–8.7 7.2 (average)	58.5–67.9 63 (average)	[41]
Lombardy, Italy	300 m^2	Year-round	6 (winter) 16 (summer) 13 (average)	–	57.2 (Dec) 74.15 (May)	[6]
Ravenna, Italy	11 m^3, 5 m^3, 1 m^3; 458 m^2 of joined RW area	Year-round	7.6	12	62	[7]
Vera Cruz, Mexico	10 m^2	Year-round	8–19	8–14	50–65	[48]
Inner Mongolia, China	28,000 to 93,000 m^2 of joined RW area	165 days production period	5.3–9.1	–	–	[26]
Inner Mongolia, China	660 m^2	July	11.2	7.5–20	50–70	[22]
Thuwal, Saudi Arabia	1m^3; 10m^3; 25 m^3 1000 m^2 of joined RW area	June–January	41.3–60.4	–	37 % (summer) 57 % (winter)	[29]

Data availability

Data will be made available on request.

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