Cultivation of Osmundea pinnatifida (Hudson) Stackhouse in the Algem® Photobioreactor System

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Abstract

Cultivation of *Osmundea pinnatifida* (Hudson) Stackhouse in flasks and/or tanks has yet to be developed beyond the lab scale. However, establishment of a methodology for the supply of this red seaweed has great potential, considering that it can be exploited either as food or as a source of biologically active compounds with potential nutraceutical, pharmaceutical and cosmetic applications. This study investigates the possibility of growing *O. pinnatifida* in the Algem® photo bioreactor (PBR) system, normally utilised for microalgae cultivation, and examines the antioxidant content of the biomass. The cultures were incubated in the Algem® PBRs under a set of environmental parameters established in a previous seasonality study at a single location in the west of Scotland. The growth of the cultures in the PBR was monitored, the biomass assessed for antioxidant content, and results compared with seasonal samples, to assess how the PBR system affects the biochemistry of the species. Analyses centred on antioxidant activity and included Total Phenolic Content (TPC) and Ferric Iron Reducing Antioxidant Power (FRAP). A significant increase in the antioxidant content, two to five-time higher compared to wild samples, was achieved by cultivating the biomass in the PBR. This study highlights that the production of antioxidant compounds in *O. pinnatifida* can be improved by increasing photoperiod and light intensity and manipulating the wavelength. This information provides important insights into how cultivation conditions for this species can be tailored to increase production and improve the composition of the final product.

1. Introduction

Macroalgae grow in complex habitats often exposed to extreme environmental conditions (such as changes in salinity, temperature, nutrients, desiccation stresses, exposure to ultraviolet radiation, etc.) (Lüning 1990). As they are subjected to combinations of high oxygen concentrations and sunlight, the formation of free radicals and other reactive oxygen species can be induced (Cruces et al. 2016). However, some seaweeds have adapted to these extreme conditions with various mechanisms and can produce a variety of bioactive and antioxidant compounds that help them mitigate against these stresses (Mišurcová 2011). In particular, secondary metabolites produced by red seaweeds, act as protectants against high irradiance and biotic stresses but also as grazing deterrents (Thomas and Kim 2011). These antioxidant components have also attracted interest for numerous commercial applications such as in the health, nutritional, cosmetic, and pharmaceutical sectors (Kim and Wijesekara 2010).

Osmundea pinnatifida (Hudson) Stackhouse is particularly exposed to environmental stresses as it grows on open rock surfaces, confined to the eulittoral-intertidal zone of sheltered to exposed coasts, often covering damp slopes, and changes its morphology and colour according to the position on the shore. It is present all year round, but it shows the greatest growth from December through to May in the UK (and has a dark colour with reduced biofouling). The texture of the biomass is generally thick and cartilaginous in nature. From June to September, it grows in creeping mats or turfs across the surfaces of rocks in the intertidal zone. Prostrate axes of the seaweed continue to grow outwards, increasing the size of the turfs, attached by closely interwoven stolonous (i.e. horizontal branch from the base) type

holdfasts (Maggs and Hommersand 1993). Erect fronds develop from October onwards, reaching their maximum size in February through to June. Although classified as red seaweed, *O. pinnatifida* can appear in a variety of colours: from black or brownish purple through to bleached yellow, with apices appearing greenish-brown in transmitted light. Fronds appear severely bleached and often damaged in early summer, because of the exposure to high temperature/light irradiance especially at low tides (Prathep et al. 2003).

Wild-harvested and dried biomass is already marketed by a few companies in the UK and Ireland as a food seasoning with a unique peppery taste, but there is also research potential in pharmaceutical and nutraceutical fields. Its biochemical composition may be particularly valuable, with various secondary metabolites reported with interesting properties (see review Silva and Pereira 2020) and that may be linked to the taste/flavour of the species (Biancacci et al. 2021).

Cultivation for this species in flasks and/or tanks is still in its infancy and few studies have investigated its on-land cultivation (Silva et al. 2019; Biancacci 2019). Traditional wild harvesting of seaweed biomass raises sustainability issues and cultivating a commercially interesting species could reduce the pressure on the wild stocks, and possibly provide a more tailored/higher-quality product. This would be an important step toward the commercial exploitation of this species, considering that obtaining substantial amounts of biomass for large-volume products is unlikely to be feasible with such a slow-growing species (Biancacci 2019).

Cultivation systems for algae range from open to closed systems, such as photo-bioreactors, the latter being used mainly for microalgal cultivation. Photo-bioreactors ranging from laboratory to industrial scale attract interest because they offer a controllable environment for conditions such as pH, CO_2, temperature and light, compared to, e.g. open pond systems (Singh and Sharma 2012). Cultivation in photo-bioreactor systems (PBR) has also been demonstrated to increase the yield of biomass and control contamination in microalgae cultivation (Ugwu et al. 2008). To date, a range of PBR types has been employed for the production of various algal products (mainly from microalgae) primarily at a laboratory scale (Ugwu et al. 2008) and it is interesting to explore whether this technology can be applied to the cultivation of seaweeds, especially the smaller macroalgal species.

The Algem® unit is a lab-scale photo-bioreactor (PBR) used for microalgae cultivation. Small-scale trials in the photobioreactor Algem® have been previously conducted for comparison of growth and production of modified strains (e.g., *Phaeodactylum. Tricornutum*, D'Adamo et al. 2018), cultivation of Cyanobacteria (e.g., *Synechocystis* sp., Lea-Smith et al. 2013), *Dunaliella* spp. for potential commercial production of carotenoids (Xu et al. 2016; 2018), strains of diatoms (Matthijs et al. 2018), *Chlamydomonas reinhardtii* strains (Stoffels et al. 2017; Young and Purton 2018), microalgae for nutrient removal (Whitton et al. 2018), and *Tetraselmis* spp. (Pereira et al. 2018). However, to our knowledge, this work is the first where this type of bioreactor has been employed for macroalgae cultivation and the targeting of interesting compounds from *O. pinnatifida*.

In this study, the Algem® PBR system has been employed to examine the impact of cultivation parameters on the growth of *O. pinnatifida*, using a range of light profiles relevant to the seasonal conditions for a particular area on the west coast of Scotland. Antioxidant capacity of the cultured biomass is assessed as a proxy of metabolic variation and biochemical adaptation.

2. Material And Methods

2.1 Samples collection and pre-treatment

Wild samples of *Osmundea pinnatifida* (whose identity was confirmed through observation of the key characters by light microscopy and molecular barcoding (Biancacci 2019)), were collected monthly at low tide from Dunstaffnage Bay (56°27'06.7"N 5°26'44.6"W) on the West coast of Scotland, over a one-year period from August 2016 until July 2017 (Biancacci et al. 2022) and used for comparison with cultivated samples in the PBR system. Biomass samples for the cultivation experiments were collected in November 2017 at low tide from Seil Island (Scotland, 56°17'17.2"N 5°38'04.2"W), where higher quantities of the species are easily accessible. Entire specimens without holdfast were obtained and then carried to the laboratory in cool boxes, cleaned manually of any visible epiphytes and/or grazers, rinsed with filtered and UV sterilised seawater three times and blotted-dry with paper towels. The samples were then treated with a 0.5% potassium iodide (KI, Sigma Aldrich®) solution in filtered seawater (FSW), to eradicate epiphytes and grazers (Biancacci 2019). They were then acclimated prior to the cultivation trials in a 20 L tank with filtered seawater and no nutrients added for one week at 10°C, 12:12 light-dark cycle at 80–100 µmol photons $m^{-2}s^{-1}$.

For biochemical analyses, the harvested biomass and the final biomass of the experiments was wrapped in aluminium foil, flash-frozen in liquid N_2 and stored at -80°C for at least 24 h. Samples were then freeze-dried and ground with a mortar and pestle (particle size < 2–3 mm) and stored at -80°C prior to analyses. Wild samples from each time point were analysed in triplicate and treated as technical replicates.

2.2. Algem® photo-bioreactor system characteristics

The Algem® (Algenuity, Stewartby, UK) is a flexible and innovative PBR designed for the cultivation of microalgae species (Fig. 1). It consists of two chambers per unit, with one flask in each chamber, which can be linked together. The flasks are closed with hermetic lids with inlets for filtered gas supply and the chambers can be independently regulated for temperature, light levels, and shaker speed. Light is provided by LEDs, blue, red, and white, with both the intensity and light cycle fully controllable. Each chamber is gently rotated by a gimbal mixing system. The temperature is controlled in the chamber by a heating/cooling system, and a temperature sensor in each chamber. The Algenious® software controlling the system can also generate modelling of monthly light and temperature profiles according to the geographical coordinates chosen. The system also provides real-time data on the pH and the growth/culture density (via absorbance) of the cultures.

The coordinates selected for the experiment were from Dunstaffnage Bay, the same area used for previous seasonal studies (Biancacci et al. 2021, 2022). Two different sets of conditions were simulated in the Algem®: one comparing winter vs summer conditions, the other comparing autumn vs spring conditions using the light quality and quantity for these months.

2.3 Comparison of summer and winter conditions on the vegetative growth of *O. pinnatifida*

For the winter vs summer experiment, December and May were chosen as months of reference, respectively, based on the results obtained from the previous seasonal study (Biancacci et al. 2022). Light conditions for the summer and winter trials are listed in Table 1 and total light is represented in Fig S1. Even if the set-up of the system includes this feature, due to technical issues the blue light was set to 0. Hence, resulting in a different total light compared to the natural light. After one week of acclimation as described above, *O. pinnatifida* specimens were blotted dried, and five grams (± 0.05 g) wet weight placed into 1 L flasks with 500 mL of sterilized F/2 culture medium (Guillard and Ryther 1962, Supplementary Table 1) for a final density of 10 g L^{-1}. Each experimental condition was replicated in triplicate with three separate cultures cultivated in the PBR for each condition. The temperature was set at 10°C for all conditions. During the experiment, the pH, light (photoperiod and intensity) and water temperature of the cultures were monitored. The samples were blotted dried and weighed and assessed for growth weekly when the F/2 medium was changed. After each medium change, the material was returned to clean flasks and incubated again in the same conditions in the PBR. The experiment ran for four weeks.

2.4 Comparison of autumn and spring conditions on the vegetative growth of *O. pinnatifida*

In a separate experiment, autumn and spring conditions were compared against a control (one control was run in triplicate for each condition, i.e., two sets of control with 3 replicate each, and then the two sets averaged for statistical analyses, since they were not statistically different). September and March were chosen as reference months, respectively, based on the previous seasonal study (Biancacci et al. 2022). Light parameters for the three conditions are listed in Table 1 and total light is represented in Fig S1. The controls were set at 10°C and a 12:12 photoperiod with a constant light intensity set at 100 µmol photons $m^{-2}s^{-1}$ (reflecting the incubation conditions used previously in experimental trials for this species, Biancacci, 2019). As above, due to technical issues the blue light was set to 0. For this trial, the temperature followed the seasonal profiles for the selected area (September max 17°C min 8°C; March max 13°C min 6°C) replicated by the PBR and referred to the air temperature rather than water temperature as this was the parameter that could be manipulated in the profiling system and software of the PBR. Three replicates were set for each condition (either autumn/spring or control): 10 g (± 0.05 g) wet weight were cultivated in 1 L flasks in 500 mL of F/2 culture medium; light, pH and water temperature were monitored during the experiment. The starting biomass was set at 10 g to increase the culture density (i.e., 20 g L^{-1}), compared to the previous experiment, in order to assess if this might affect the growth rate. The samples were blotted dried and weighed weekly when the F/2 medium was changed as

described above. The experiment ran for a total of five weeks. The length of the experiment was extended for a further week, compared to the previous trial, to assess if the trend of the growth changed or remained constant.

Table 1

Summary of light wavelength and intensity (expressed as µmol photons $m^{-2}s^{-1}$) and photoperiod for all the 5 conditions trialled in the PBR system in the two experiments

Condition	Red light (µmol photons $m^{-2}s^{-1}$)		White light (µmol photons $m^{-2}s^{-1}$)		Total light (µmol photons $m^{-2}s^{-1}$)		Photoperiod	
	Max	Average	Max	Average	Max	Average	Hr Light	Hr Dark
Summer	170	70	920	380	1090	450	17	7
Winter	30	5	170	31	200	36	7	17
Autumn	100	31	545	180	645	211	13	11
Spring	90	30	500	151	590	181	13	11
Control	n/a	n/a	100	100	100	100	12	12

2.5 Physiology and growth assessment

Pulse-Amplitude-Modulation (PAM) Fluorometry was applied to assess in a non-intrusive way the physiological response of the cultures to the incubating conditions measuring the health (e.g., efficiency) of the photosystems. An AquaPen-P® (Photon Systems Instruments, PSI) hand-held PAM fluorometer for measurements of photosynthetic parameters in algae was employed according to manufacturer instructions, at the beginning of the experiment and weekly. The results were expressed as quantum yield (QY), equivalent to Fv/Fm (ratio of variable fluorescence-Fv- on maximum fluorescence-Fm) in dark-adapted samples. Briefly, the fluorometer was placed on the dry surface of the seaweeds (after brief acclimation in the dark), the excitation pulse flashed, and the response of the photosynthetic efficiency of the alga to the absorbed light re-emitted as fluorescence was recorded by the instrument as Fv/Fm. The measurements of the maximum quantum yield were taken three times in different parts of the thalli and averaged. The starting QY average for the samples was around 0.40–0.50 (Fv/Fm).

Growth was estimated through weight measurements. The samples were blotted and then weighed when the F/2 medium was changed every week. Specific growth rate (SGR, % day^{-1}) was assessed using the formula according to Evans (1972):

$$SGR\left(\%day^{-1}\right) = \frac{(LN(Wt) - LN(W0))}{t} \times 100$$

Where LN is the natural logarithm of *Wt*, the total seaweed biomass at a certain time minus *W0*, the initial seaweed biomass at time 0, and *t* is the time (in days).

2.6 Total Phenolic Content and Antioxidant Capacity

At the end of the experiment, the biomass was freeze-dried and milled for further analyses and comparison with wild-harvested biomass. Samples (100 mg ± 5 mg) were extracted with 1 mL of 50% acetonitrile/ ultrapure water (UPW) containing 0.1% (v/v) formic acid (Biancacci et al. 2021, 2022). After centrifugation (16,100 g at 5°C for 10 minutes), the supernatant extracts were transferred to new tubes, completely dried in a Speed Vac rotatory evaporator and then re-suspended in 600 µL of 20% acetonitrile/UPW, vortexed and placed in an ultrasonic bath until fully re-suspended. They were then centrifuged at 16,100 g (13,200 rpm) for 10 minutes at 5°C.

The total phenolic assay was performed applying the Folin-Ciocalteu method modified from Vuong et al. (2014). To 10 µL of extract, 240 µL of UPW and 250 µL of Folins reagent (Sigma Aldrich®) were added, mixed and the samples were left to stand for 3 minutes. Then 500 µL of filtered (Whatman® filter paper, pore size 11µm) 13% (w/v) sodium carbonate was added, mixed and samples were left to stand for one hour at room temperature. The absorbance was read at 750 nm in an Ultrospec 3100 pro (UV/Visible spectrophotometer) against blanks. Phloroglucinol (PG) was used as the standard for a calibration curve and the results were expressed as µg PGE mg^{-1} DW.

A FRAP assay modified from Benzie and Strain (1999) was also performed on the extracted samples as a measure of antioxidant capacity. The reagents used for the reaction were: 300 mM sodium acetate buffer, pH 3.6; 10 mM 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl; 20 mM $FeCl_3 \cdot 6H_2O$ in UPW. 214 The working solution was made by mixing 50 mL of acetate buffer with 5 mL of TPTZ and 5 mL of ferric chloride solution. 100 µL of sample was mixed with 900 µL of the working solution and read together with a blank and the standards 4 min after the addition of the working solution, in an Ultrospec 3100 pro (UV/Visible spectrophotometer) at 593 nm. Iron (II) sulphate was used as the standard for the calibration curve (between 100–1000 µM) and the results were expressed as µM $FeSO_4$ eq mg^{-1} DW.

2.7 Statistical analyses

All values are averages of triplicate samples ± standard deviation (SD). All data were tested for normality prior to ANOVA analyses (Kolmogorov-Smirnov normality test) and equivalence of variance (Levene's test, $P > 0.05$). The significant differences in growth and biochemical composition among the treatments were determined by One-way analysis of variance per each component (One-way ANOVA, $P < 0.05$, Minitab 18). Statistically significant interactions were further analysed using a post hoc test (Tukey's HSD) to determine the sample main effects. Significance was set at the 5% level ($\alpha = 0.05$).

3. Results

3.1 Comparison of summer and winter conditions on the vegetative growth of *O. pinnatifida*

The specific growth rate (% day⁻¹) varied from − 0.25 to 0.5 under summer conditions (corresponding to a maximum increase in weight of 0.03 g per day) and from − 0.3 to -0.6 under winter conditions, with positive values recorded for the summer conditions in the second and third weeks, whilst overall the cultures under winter conditions showed a slow decrease in their SGR (Fig. 2a). The increase in growth in the summer samples was statistically different compared to the winter samples ($F_{1,22}$=4.65, P = 0.042, Fig. 2a). In fact, the winter samples did not show any significant growth, although they were alive (as assessed by QY data and morphological assessment). A difference in the colour of the cultures depending on the conditions applied was also recorded (Supplementary Fig. 2), where cultures incubated in summer conditions were paler and with brown tips while cultures incubated under winter conditions were of a dark red colour, which reflects what is observed in wild specimens (Biancacci et al. 2022).

The average pH was 8.90 ± 0.5 SD for the summer simulation and 8.52 ± 0.3 SD for the winter. QY was measured weekly, and no statistical difference was noted between the two conditions ($F_{1,28}$=3.17, P = 0.09, Fig. 2b). However, after an initial decrease in the QY values, the cultures under winter conditions showed an increase in the photosynthetic response probably due to the acclimation of the cultures to the incubation conditions and a less stressed physiological status compared to the cultures under summer conditions.

3.1.2 TPC and Antioxidant capacity

The total phenolic contents (TPC) of seaweed material grown in the PBR were higher than the TPC measured on seasonal samples collected from Dunstaffnage beach for those months. The TPC of PBR summer cultivated material was 174.1 ± 13.3 µg mg^{-1} and 142.0 ± 15.9 µg mg^{-1} for the PBR winter material, both significantly higher than the wild-harvested material (i.e. May 92.7 ± 17.4 µg mg^{-1} and December and 92.5 ± 17.1 µg mg^{-1}, One-way ANOVA, $F_{3,8}$=18.75, P = 0.001, Fig. 3a). There was no statistically significant difference between the winter and summer TPC levels in the PBR-cultivated samples (P > 0.05, Tukey's HSD tests).

The FRAP values of the PBR-cultivated samples (winter and summer) were statistically higher than the wild-harvested samples (One-way ANOVA, $F_{3,8}$=83, P < 0.001, Fig. 3b). The summer PBR cultivated material had a FRAP value of 5.0 ± 0.8 µM mg^{-1} and the winter PBR cultivated material was 3.3 ± 0.2 µM mg^{-1}. This difference between summer and winter cultivated samples was significant at P < 0.05. The FRAP values of the wild seasonal samples were significantly much lower (December 0.43 ± 0.08 µM mg^{-1} and May 0.96 ± 0.08 µM mg^{-1}).

3.2 Comparison of autumn and spring conditions on the vegetative growth of *O. pinnatifida*

The growth rate (% day⁻¹) varied from a minimum of 0.02 to a maximum of 0.5 in spring, from a minimum of -0.33 to a maximum of 0.40 in autumn and from a minimum of -0.18 to a maximum of 0.40 for the control experiments (Fig. 4a).

There were no statistical differences observed between growth rates under the three conditions (One-way ANOVA and $F_{2,42}$=0.34, P = 0.717 Fig. 4a). The growth rate was mostly positive throughout the experiment and the cultures looked healthy, as in the previous trial, and new growth was observed (Supplementary Fig. 3).

The average pH was 8.25 ± 0.6 SD for the spring and pH 8.43 ± 0.5 SD for the autumn simulation and pH 8.90 ± 0.5 SD for the control. There was a statistically significant difference for the QY values between the three conditions in the first and last weeks (September > Control > March, One-way ANOVA $F_{2,6}$=10.9, P = 0.010 and $F_{2,6}$=9.32, P = 0.014, respectively, Fig. 4b), showing an initial stress in the cultures due to the new incubation conditions and the gradual adaptation of the cultures, particularly for those under autumn incubation, towards the end of the experiment.

3.2.1 TPC and Antioxidant capacity

The highest TPC was recorded in autumn PBR samples (270.60 ± 28.9 µg mg^{-1}), followed by spring PBR (243.02 ± 29.20 µg mg^{-1}) and control (215.90 ± 15 µg mg^{-1}) samples. However, differences were not significant between the treatments (Fig. 5a). On the other hand, cultivated samples were significantly higher than the wild samples for both tested conditions (autumn and spring, One-way ANOVA $F_{4,10}$=35.6, P< 0.001). The same trend was noted for FRAP analyses, with the highest FRAP value observed in the autumn PBR samples (4.50 ± 0.43 µM mg^{-1}), which were statistically different from the control and the wild seasonal samples, followed by the spring PBR and the control (4.14 ± 0.34 µM mg^{-1} and 3.62 ± 0.20 µM mg^{-1}, respectively) which were both statistically different from the wild seasonal samples (One-way ANOVA $F_{4,10}$=101.8, P< 0.001, Fig. 5b).

4. Discussion

The Algem® system is currently marketed as a PBR for microalgal cultivation with the possibility to reproduce, in a controlled situation, environmental parameters of a specific geographical area. This represents the possibility to determine the impact that environmental factors have on both biomass productivity and biochemical composition. Previous research on the cultivation of microalgae in the Algem® has mainly used the system as a controlled environment for cultivation trials, without focusing on effects on the biochemical profiles of the species. Nevertheless, all the species of microalgae cultivated in this PBR were investigated for their potential future commercial/ biotechnological applications as sources of carotenoids, secondary metabolites, antibacterial compounds or nutrient removal for waste-water treatments (D'Adamo et al. 2018; Lea-Smith et al. 2013; Xu et al. 2016, 2018; Matthijs et al. 2018; Stoffels et al. 2017; Young and Purton 2018; Whitton et al. 2018). In a similar study

to the one reported here, Pereira et al. (2018) investigated how the simulation of seasonal conditions might affect the growth of *Tetraselmis* sp., observing statistical differences in the growth rate of the selected species for the simulated spring season compared to the autumn.

The feasibility of using this PBR system for small-scale *in vitro* cultivation of this seaweed was demonstrated for all the treatments tested, except for the winter conditions. Nonetheless, the growth was slow, with a maximum SGR (% day^{-1}) of around 0.50. With the limitations that the winter and summer conditions were tested in a separate trial compared to the spring and autumn conditions, overall, the results reported suggest that an initial density of 20 g (FW) L^{-1} and culturing conditions set for spring, control, or autumn, would results in better growth, compared to the 10 g (FW) L^{-1} and summer or winter conditions trialled. In addition, results suggest that lower temperatures together with long daylight photoperiod (> 12h) might play a synergic role in the growth of this species, where the control and autumn and spring light conditions and temperatures might have played a role in the better performance of the cultures, compared to summer and winter.

The photosynthetic quantum yield (QY) is a biophysical parameter that can provide information on the physiology (e.g., photoinhibition, recovery and acclimation) and overall performance of the seaweeds, looking at the photochemical efficiency of light utilization. Previous studies have reported Fv/Fm values of around 0.5 or lower (Liu and Pang 2010) for red algae in cultures, which is similar to what observed in this study. The QY increased across the cultivation, after an initial decrease (presumed to be due to the response of the seaweeds to the new cultivation conditions) as the species adapted to the incubation conditions. The alga responded as it would under similar conditions in the wild for example, the changes in colour that the cultures underwent, especially in the summer simulation, were the same reported in the wild samples harvested during the same relevant season (Biancacci et al. 2022).

Certain seaweed properties are dependent or can be influenced by environmental parameters (Lüning 1990; Lobban and Harrison 1994), such as temperature, light regime, photoperiod, etc., with the potential to produce interesting compounds in artificial conditions. Several studies have focused on the antioxidant properties of species belonging to the *Laurencia* complex, including *O. pinnatifida*, investigating the capacity of the seaweed to produce secondary metabolites of antimicrobial value. These include volatile components, phenols, terpenes (Ozdemir et al. 2004; Xu et al. 2003; Karabay-yavasoglu et al. 2007; Vairappan et al. 2003), steroids (Awad 2000), phlorotannins (Nagayama et al. 2002) and lipids (Freile-Pelegrini and Morales 2004). Edible seaweeds can also contain appreciable amounts of polyphenols (Rodriguez-Bernaldo de Quiros et al. 2010), which can be effective antioxidants and may have interesting biological activities (e.g. inhibition of proliferation of cancer cells, Kwon et al. 2007; Yuan et al. 2005a; influence in anti-inflammatory responses, Kim and Wijesekara 2010; influence on response to diabetes, Lee et al. 2010). Since the 1980's, red algae, including *O. pinnatifida*, have been investigated for the production of terpenoid compounds, which have pharmaceutical/nutraceutical applications (Iliopoulou et al. 2002; Suzuki et al. 2002). Increasing the production of antioxidant compounds by manipulating cultivation conditions is particularly important due to the potential commercial exploitability of these extracts for cosmetic, pharmaceutical and/or nutraceutical

applications (Yabuta et al. 2010; Zhang et al. 2003; Munir et al. 2013). Indeed, the global market for antioxidants is profitable and it is constantly growing, (Kaur and Das 2011) and numerous algae have been already identified for their potential as functional food ingredients (Holdt and Kraan 2011; Freile-Pelegrín and Robledo 2013).

Cultivating *O. pinnatifida* in the Algem® bioreactor has demonstrated that employing this system has the potential to enhance the antioxidant compound production. Higher antioxidant capacity and total phenolic content in cultivated samples compared to wild-harvested samples were demonstrated in all the trials conducted, where the TPC was doubled, and the antioxidant capacity was ~ five-fold higher. These values were higher than the highest values previously reported for the wild-harvested samples (127.5 ± 2.2 µg mg^{-1} TPC and 2.04 ± 2.2 µM FeSO4eq mg^{-1} FRAP in July, Biancacci et al. 2022). In addition, the ratio of the FRAP to TPC values was consistently increased in the cultivated samples over the wild-harvested samples. There are often correlations between FRAP and TPC values within extracts from one sample species (e.g., berries – Deighton et al. 2000), which is probably because both the Folin reaction and the FRAP assay are electron transfer-based assays and measure similar aspects of antioxidant activity (Huang et al. 2005). Therefore, when the ratio between these measures varies considerably it suggests a substantial alteration in the metabolic composition of the tissue, possibly in response to the quantity but also to the quality of light used. This observation is important for further commercial applications. As already demonstrated for other species, antioxidants often include compounds that are used by the seaweeds to protect themselves from light irradiation, photo-oxidation, UVB irradiations, etc. (Munir et al. 2013). It is not surprising that the controlled manipulation of the light parameters under specific cultivation conditions trialled had an impact on the production of such compounds, significantly increasing their content. In addition, the F/2 medium in which the seaweeds were cultivated could have also had an effect in increasing the antioxidant content due to the presence of compounds that have previously shown antioxidant activity (e.g., Vitamin B_{12}, van de Lagemaat et al. 2019); however, the medium was not texted for antioxidant response in this study. Understanding the metabolic changes that underlie these variations in TPC and antioxidant capacity could provide a mechanism for producing a more commercially valuable product that could be sold as a delicacy at a higher price than the wild-harvested biomass to restaurants or exploited for applications in the pharmaceutical and nutraceutical sectors.

Ideally, *O. pinnatifida* could be cultivated in winter, when antioxidant compounds are lower than in summer (Biancacci et al. 2022) and the concentration of commercially interesting antioxidant compounds could be efficiently improved in the PBR system by tailoring light, photoperiod, and nutrient conditions. Light and temperature parameters applied in the autumn and spring simulations resulted in the highest antioxidant concentration, although the densities were different between experiments. The increase in total phenolic content observed from 100 µg mg^{-1} DW in wild samples to 270 µg mg^{-1} DW in cultivated samples in only four weeks with corresponding increases in FRAP from 0.82 µM mg^{-1} to 5 µM mg^{-1} is substantial. It is interesting that the cultures grown under control conditions also achieved higher levels of antioxidant compounds than the wild-harvested samples. This suggests that cultivating the

species in flasks and/or tanks under the ideal parameters identified in the control conditions (Biancacci 2019), which are similar to the autumn and spring light conditions trialled, would significantly increase the content of antioxidant compounds. Alternatively, the high content reported for the cultures under control conditions might be a result of the long daylight photoperiod (12:12) associated with white light, which could be the driving parameter (being high and prolonged also in the autumn and spring conditions, i.e., photoperiod 13:11) for the recorded increased in antioxidant content.

While the PBR system cannot be used for the production of biomass in large-scale systems, this methodology has the potential for low-volume high-quality production, focusing on the final high concentration of antioxidants, or other interesting bioactive components. This can off-set increased costs of cultivation in a PBR, when taken into consideration with the commercial value of the final product.

5. Conclusions

Osmundea pinnatifida can be cultivated in the Algem® unit, producing healthy cultures, albeit with a slow growth rate, but cultures were healthy with improved antioxidant content. Morphology and colour of the samples cultivated in the unit matched that of the wild-harvested samples for the same months of reference. This study also demonstrated that is possible to use this system to target commercially interesting properties not only for microalgae cultivation, but also for the production of macroalgae, giving the opportunity for commercial applications not only in the established food market, substituting for the wild-harvested product, but also in nutraceutical and pharmaceutical sectors.

Declarations

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Conflict of Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Data availability

The authors confirm that all relevant data supporting the findings of this study are included in the article and its supplementary information files.

Code availability

Not applicable

Authors' contributions

Cecilia Biancacci: Conceptualization, Methodology, Validation, Formal analyses, Investigation, Writing - Original Draft, Writing - Review & Editing, Visualization **Gordon J. McDougall**: Writing - Review & Editing, Supervision, Resources **John G. Day:** Writing - Review & Editing, Supervision, Resources **Michele S. Stanley**: Conceptualization, Writing - Review & Editing, Supervision, Resources, Project Administration, Funding acquisition

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Figures

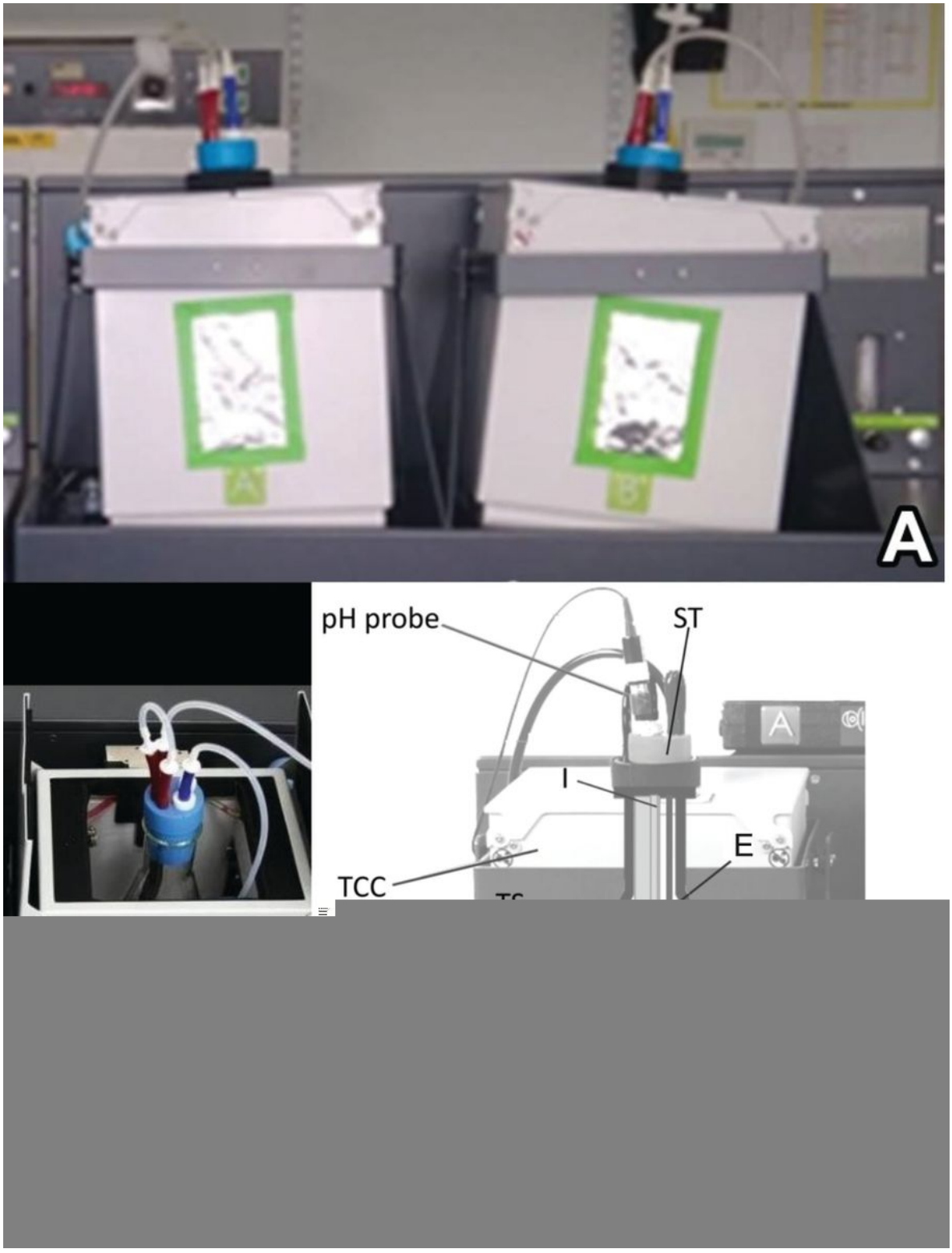

Figure 1

A) The Algem® photobioreactor used for the experiment; **B)** View of the temperature controlled chamber from above with a 1L conical flask inside; **C)** Schematic of the PBR system: ST= stopper, I= influent, E = effluent, TCC = temperature controlled chamber, MP= micro-processor, TS= Temperature sensor, H= heating system, C=cooling system, V=valve box, CH= chiller, GMP=Gimbal mixing system

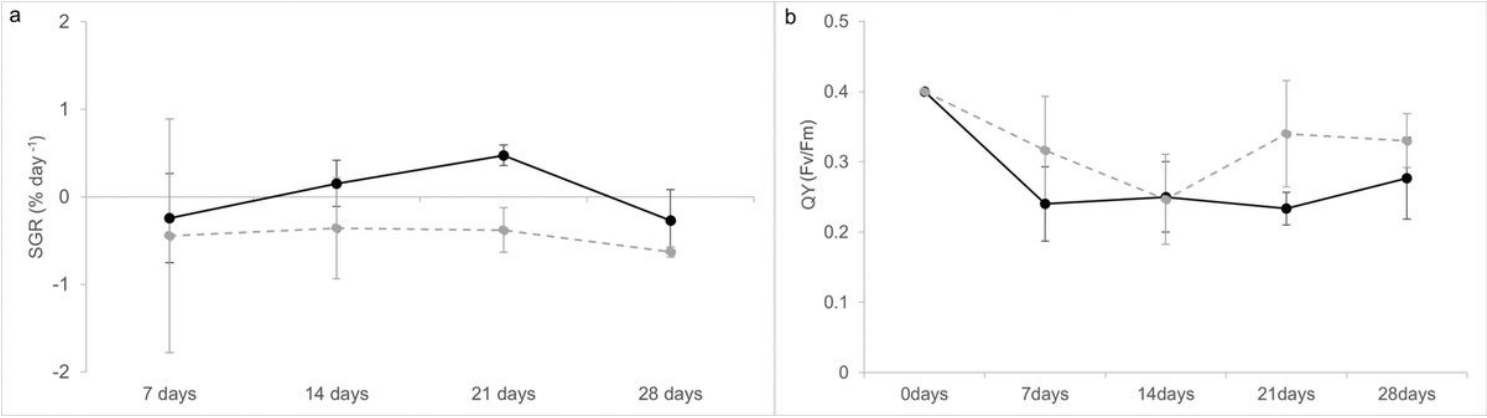

Figure 2

A comparison of **A)** SGR (% day^{-1}) and **B)** Quantum Yield (QY as Fv/Fm) measurements of cultures under summer (black bars) and winter (dashed grey bars) conditions in the PBR (n=3; error=SD)

Figure 3

Comparison of **A)** TPC (µg mg^{-1}) and **B)** FRAP (µM mg^{-1}) between field (striped bar) and PBR cultivated samples (plain bar) in relation to summer and winter conditions (n=3; error bar= SD). Letters above bars refers to significant differences (Tukey's HSD, P<0.05)

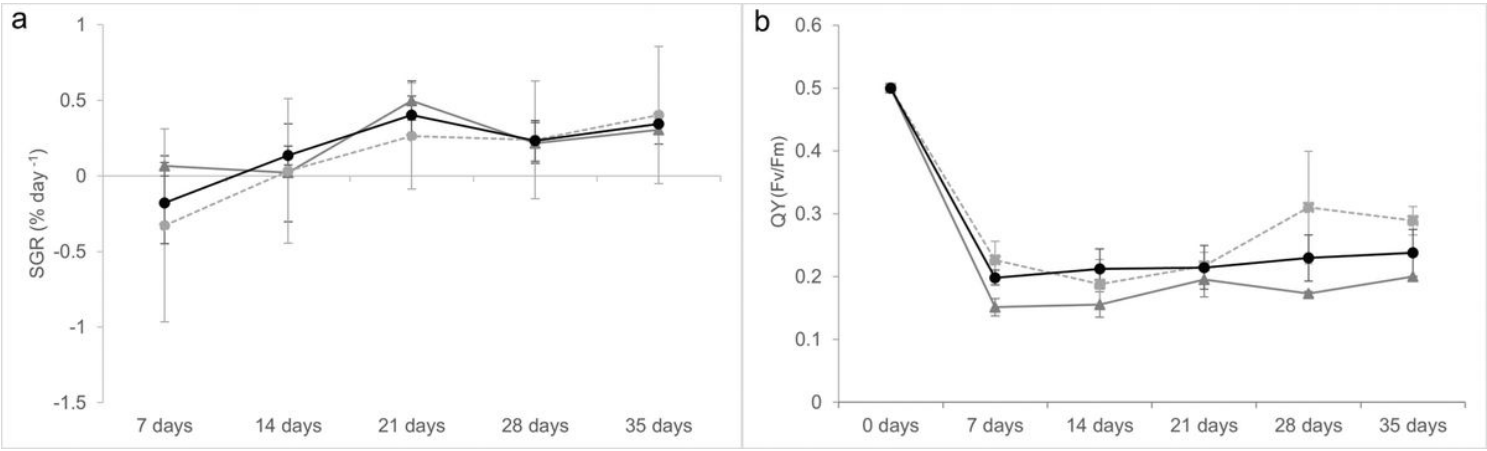

Figure 4

A comparison of **A)** SGR (% day^{-1}) and **B)** Quantum Yield (QY as Fv/Fm) measurements of control (black line), spring (grey line, triangle marks), and autumn (dashed grey line, square marks) over 5 weeks (n=3; error bar=SD)

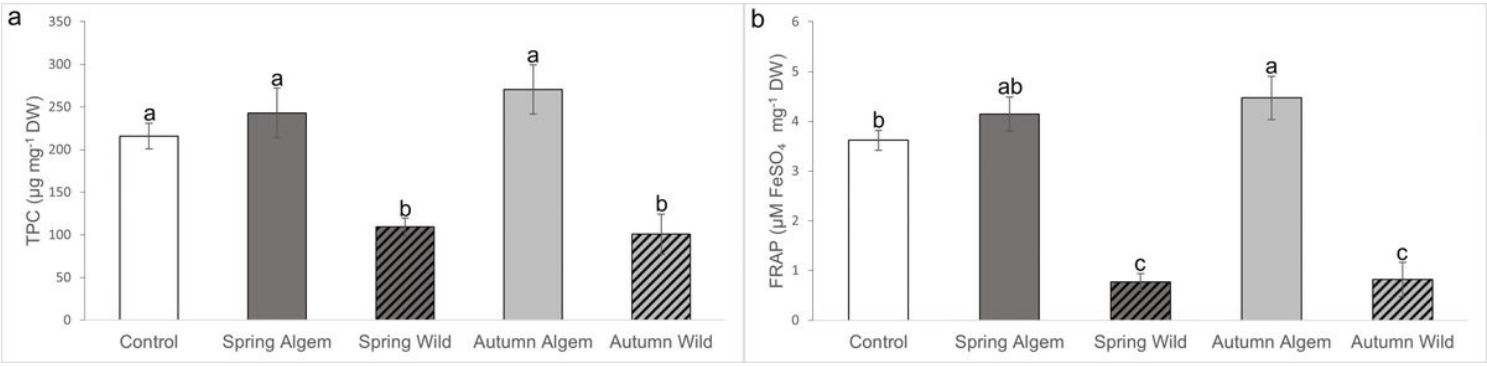

Figure 5

Comparison of **A)** TPC (µg mg^{-1}) and **B)** FRAP (µM mg^{-1}) between control (white bar), field (striped grey bar) and PBR cultivated samples (plain grey bar) in relation to spring and autumn conditions (n=3; error bar= SD). Letters above bars refers to significant differences (Tukey's HSD, $P<0.05$)

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