



Effects of light quality and intensity on growth and bromoform content of the red seaweed *Asparagopsis taxiformis*

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Received: 23 June 2023 / Revised: 22 July 2023 / Accepted: 25 July 2023 / Published online: 9 August 2023
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Abstract

Species of the genus *Asparagopsis* are rich in halogenated bioactive compounds, particularly bromoform. Its use as a feed additive in ruminant livestock drastically decreases the animal's methane production, thereby reducing the industry's environmental impact. Addressing the high demand for *Asparagopsis* biomass requires the understanding of the culture conditions that promote higher growth rates and bromoform content. Here we evaluated how different light quality combinations (High-Blue:Red, Medium Blue:Red, High-Blue:Green:Red, and White) and four light intensities (30, 60, 90 and 120 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) affect the growth and bromoform content of the *Asparagopsis taxiformis* tetrasporophyte in indoor tumbling cultures at two biomass densities. We also assessed the effect of light intensity on the photosynthetic response by measuring oxygen evolution rates. Light spectra containing intermediate wavelengths promoted higher growth, regardless of biomass density. Of the different light qualities tested, white light promoted the highest bromoform content. Increasing light intensity led to a positive response in *A. taxiformis* growth. However, the photosynthetic parameters estimated showed that the two higher light intensity treatments were above the saturation irradiance, for both culture densities. This, along with the observed development of contamination, suggests that long-term cultures of *A. taxiformis* should be maintained at light intensities no higher than 60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. In addition, we found that exposing cultures to higher irradiances does not guarantee a bromoform-richer biomass. These results offer valuable insights for optimizing biomass and bioactive compound production in indoor cultures of the *Asparagopsis* genus.

Keywords Seaweed cultivation · Light management · Bromoform · Photosynthesis · Rhodophyta · *Asparagopsis* aquaculture

Introduction

Species of the red seaweed genus *Asparagopsis* (*A. armata* and *A. taxiformis*) have the ability to produce and accumulate a high amount of diverse bioactive halogenated compounds in their cells (Paul et al. 2006). These compounds provide *Asparagopsis* biomass with anti-microbial, anti-parasitic and antitumor activities, with numerous biotechnological and industrial applications (Paul et al. 2006; Hutson et al.

2012; Mata et al. 2013; Greff et al. 2014; Pinteus et al. 2018). Recently, it was discovered that bromoform (the main halogenated compound found in *Asparagopsis*) also has anti-methanogenic activity. Supplementing ruminant diets with *Asparagopsis* at low inclusions rates (0.2–2% of the animal's daily ration weight) significantly reduced enteric methane emissions by up to 98% (Kinley et al. 2016, 2020; Machado et al. 2016; Roque et al. 2019, 2021). Methane released from ruminant digestion is the food system's single largest negative planetary impact responsible for approximately 80 million metric tons of CO₂-equivalent/year (FAO 2020). Realizing global scale climate benefits from *Asparagopsis* anti-methanogenic properties requires the development of an entirely new seaweed farming sector.

Asparagopsis is a versatile genus for aquaculture due to the heteromorphic nature of its life cycle, which allows a range of different propagation strategies (see Zanolla et al. (2022) for details on cultivation strategies for the

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different life cycle stages). In this work, we focused on the cultivation of the tetrasporophyte phase, suitable for on-shore cultivation using vertical bioreactors. The algae grow vegetatively (clonally) through apical growth and, when filaments break down, fragments develop into new clumps, allowing a continuous propagation and production of biomass, assuming all growth requirements are met. Growing the sporophyte can be a standalone production method of *Asparagopsis* biomass (Schuenhoff et al. 2006; Mata et al. 2010), as well as a source of biomass (broodstock) to induce sporogenesis and produce the spores for gametophyte cultivation in the ocean. Therefore, mastering the cultivation of the sporophyte phase is of paramount importance to produce *Asparagopsis* biomass, regardless of the chosen culture method (in- or off-shore).

Light management is a particularly valuable tool in seaweed cultivation due to its direct influence on photosynthetic activity, biomass growth, and biochemical composition (Godínez-Ortega et al. 2008; Borlongan et al. 2020; Zepeda et al. 2020; Ghedifa et al. 2021; Liang et al. 2022). The sporophyte of *Asparagopsis* can be produced both in outdoor (using natural sunlight) or indoor systems (using artificial light) (Schuenhoff et al. 2006; Mata et al. 2010; Nilsson and Martin 2022). In both cases, light can be managed by controlling the spectral composition (using filters or LED lights) and the intensity (using neutral density filters or dimmable lights), as well as by manipulating the density of the algae inside the culture vessels. In the only study that focused on the effects of light quality and intensity on *Asparagopsis* growth and morphology, Monro and Poore (2005) demonstrated that spectra with medium to high ratios of Blue:Red light promoted compact phenotypes of *A. armata* sporophyte (higher number of meristems correlated with second-order branch production) and consequently optimized growth, particularly using higher irradiance levels (40 and 60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Studies on the effects of light intensity on *Asparagopsis* sporophyte physiology have so far only evaluated the photosynthetic response through photosynthesis-irradiance curves (PI-curves) (Mata et al. 2006; Zanolla et al. 2015). The two studies suggested different photoinhibiting irradiances, with Mata et al. (2006) reporting photoinhibition at light intensities above 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, but no photoinhibition was reported in Zanolla et al. (2015) at intensities as high as 600 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. While PI-curves provide valuable information, the irradiance values used as a reference may not be directly used to optimise seaweed culture, particularly because the experiments from the above-mentioned studies were carried out in static conditions, which do not correspond to the reality of cultivation, where tetrasporophytes are cultivated in a dynamic light environment (biomass in tumbling suspension at varying densities), with variable self-shading

patterns that affect both the quality and intensity of light, regardless of the light source settings.

Light can also be used as a management tool to enhance the concentration of valuable compounds in seaweed aquaculture. To the best of our knowledge, no studies analysed the effects of light quality or intensity on the concentration of bromoform in *Asparagopsis*. There is substantial evidence that bromoform and other halogenated molecules are produced as a part of a reactive oxygen species production and manipulation cascade, which converts hydrogen peroxide to halogenated chemicals via specific halogenated enzymes (Thapa et al. 2020). Consequently, light intensity may affect the production of bromoform indirectly, as increasing irradiances may lead to oxidative stress as a result of increasing production of hydrogen peroxide and other reactive oxygen species (Dummermuth et al. 2003). On the other hand, directly adding hydrogen peroxide in *A. taxiformis* cultures resulted in physiological damage to the seaweed, inhibition of the activity of halogenated enzymes, and ultimately in a decrease of bromoform concentration in the tissue (Mata et al. 2011).

The objectives of this study were to evaluate how light quality and intensity affect biomass growth, photosynthetic performance, and bromoform content of *Asparagopsis taxiformis* tetrasporophyte, cultured in indoor tumbling conditions at two different biomass densities.

Materials & methods

Algal material, culture conditions and species identification

Samples of *Asparagopsis* tetrasporophytes were collected by snorkel from a depth range of 1–3 m at La Herradura bay, South Spain (36°43'23.4"N, 3°43'36.5"W), in October 2018. Samples were taken to the laboratory at the Centre of Marine Sciences (CCMAR, Portugal) and cleaned from visible epiphytes under a dissecting microscope. Seaweeds were cultured in transparent culture vessels with bottom aeration to keep the biomass in constant movement and maintained in a walk-in culture chamber at constant temperature and light (22 °C, 12:12 light/dark cycle, 30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), using UV-sterilized natural seawater (salinity of 33 ppt and pH of 8.2), enriched with one-half (f/2) of medium f (Guillard 1975), refreshed weekly. The biomass density was maintained between 1–2 g fresh weight (FW) L^{-1} for at least 30 months prior to the start of the experiments.

Due to morphological similarities between tetrasporophytes of the two species of the *Asparagopsis* genus (*A. armata* and *A. taxiformis*), the mitochondrial marker cytochrome oxidase subunit 2—subunit 3 (*cox2-3*) spacer (Zuccarello et al. 1999) was chosen to obtain phylogenetic

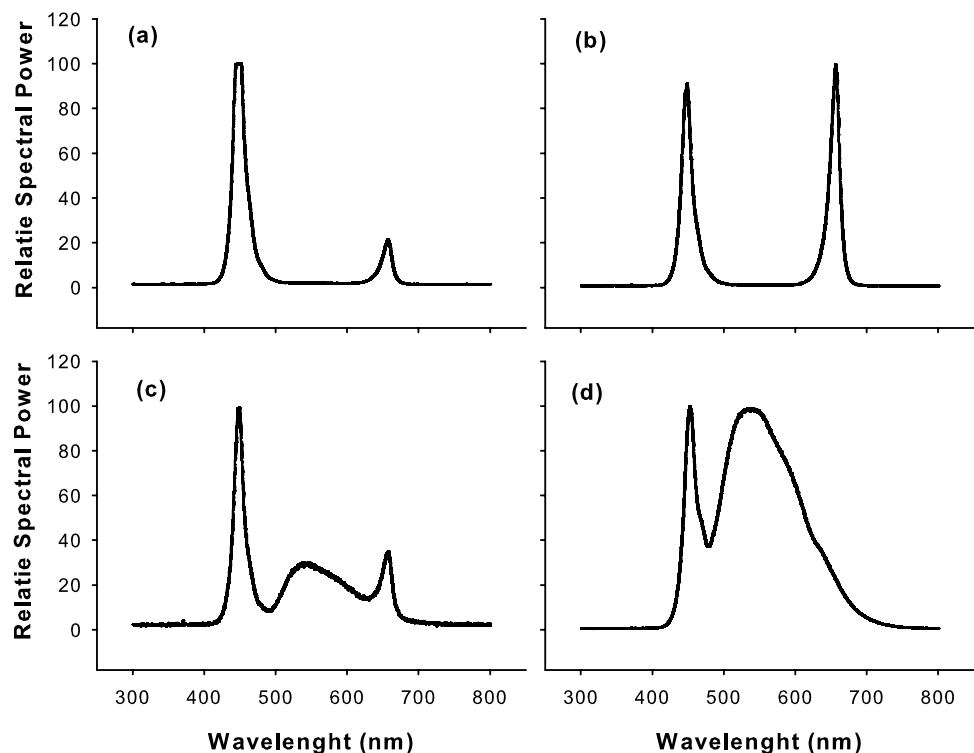
information and properly identify the species used in this study. DNA extraction and PCR-amplification were performed following procedures described in Torres et al. (2021). PCR products were sequenced in an automated capillary sequencer (Applied Biosystems, CCMAR, Portugal). Newly generated and downloaded sequences of the *cox2-3* spacer from *Asparagopsis* spp. (Andreakis et al. 2016) from GenBank were proofread, edited, and aligned in GENEIOUS v.2019.2.1 (<http://www.geneious.com>). The *cox2-3* sequence of the tetrasporophyte used in this study was similar to the Italy (AY589530) and South Africa (JF820073) specimens of *Asparagopsis taxiformis* Lineage 2 and, therefore, identified as *A. taxiformis*.

Light quality experiment

To test the effect of light quality on growth and bromoform content of *A. taxiformis*, cultures were subjected to a combination of four light qualities: High-Blue:Red (High-BR); Medium Blue:Red (BR); High-Blue:Green:Red (High-BGR); and White (W) (Fig. 1), and two biomass densities (0.5 and 2.0 g FW L⁻¹) for 3 weeks. The first week was considered as the acclimatory period so no data from that time was included in the final analysis. The different qualities of light were achieved using light emitting diodes (LEDs) (ELIXA 600W, Heliospectra), programmed using the Heliospectra System Assistant software to produce

different relative spectral distributions with equivalent total PAR (Fig. 1). Spectral distribution was measured by using a spectroradiometer (OceanOptics USB2000 + UV-VIS). For this study, blue light was considered as the 400–485 nm range, red light was 630–700 nm, and the intermediate waveband (486–629 nm) was referred to as green light, although this waveband also includes orange and yellow spectra components. For all treatments, the irradiance (PAR) was maintained constant at 30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, a level within the light-limited portion of the PI curve, as determined by preliminary tests. Experimental vials were installed inside white foamboard cabinets, to create homogenous and discrete light fields for all light sources. For each light quality treatment, five replicates ($n=5$) of 0.5 or 2 g FW L⁻¹ (depending on each biomass density treatment) of *A. taxiformis* were placed in transparent glass flasks with 1 L of UV-sterilized natural seawater (salinity 33 ppt, pH 8.2) enriched with *f/2* medium (Guillard 1975) and maintained under controlled temperature (22 °C), with a light:dark cycle of 12:12 h. Culture flasks with constant bottom aeration provided water and seaweed motion. At the end of each week, algae were weighed and returned to refreshed culture media at the experimental stocking densities. At the end of the experiment, all algae biomass was stored at –20 °C until chemical analysis. Prior to the analysis, all biomass was freeze-dried (–50 °C, 80 μbar , Labconco, USA) for 48 h and homogenized.

Fig. 1 Relative spectral distributions of the four light qualities used in this study: (a) High Blue: Red, (b) Medium Blue:Red, (c) High Blue:Green:Red and, (d) White



Light intensity experiment

To test the effect of light intensity on growth and bromoform content, *A. taxiformis* tetrasporophyte was exposed to a combination of four light intensities (30, 60, 90, and 120 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and two biomass densities (0.5 and 2.0 g FW L^{-1}) for 3 weeks. The first week was considered the acclimatory period, so no data from that time was included in the final analysis. The spectral composition was maintained at the W light quality (white light). Irradiance levels were achieved using LEDs attenuated with neutral-density light filters. White foamboard cabinets were again used to create homogenous and discrete light fields for each light source. For each light intensity treatment, five replicates ($n=5$) of 0.5 or 2 g FW L^{-1} (depending on each biomass density treatment) of *A. taxiformis* were placed in transparent 1-L glass flasks filled with UV-sterilized natural seawater (salinity 33ppt, pH 8.2) enriched with f/2 medium (Guillard 1975) and maintained under controlled temperature (22 °C), with a light:dark cycle of 12:12 h. Culture flasks were supplied with constant bottom aeration to provide water motion and supply carbon. At the end of each week, algae were weighed and returned to refreshed culture media at the experimental stocking densities. At the end of the experiment, the effect of light intensity on photosynthetic response was evaluated by measuring oxygen evolution rates. The remaining algal biomass was stored at -80 °C until chemical analysis. Before analysis, all material was freeze-dried (-50 °C, 80 μbar , Labconco, USA) for 48 h and homogenized.

Daily growth rates

Growth was assessed weekly as the difference in total biomass, over the three weeks of each experiment. Before weighting, algae were centrifuged (30 s, 1690 \times g) to remove residual water. The daily growth rate (DGR), expressed as $\% \text{ day}^{-1}$, was determined using the following formula, as recommended by Yong et al. (2013):

$$DGR = \left[\left(\frac{W_t}{W_0} \right)^{1/d} - 1 \right] \times 100$$

where W_t and W_0 are the final and initial FW mass, and d is the time in days.

Since the first week of each experiment was considered the acclimation period, DGR was reported as the mean of the second and third weeks.

Photosynthetic response

The photosynthetic response of *A. taxiformis* to light intensity was assessed through photosynthesis-irradiance

(PI) curves, by measuring oxygen evolution rates after the light intensity experiment. For each PI curve ($n=5$), 150 or 600 mg FW of *A. taxiformis* (depending on each biomass density treatment) were transferred to the measuring chamber, filled with 300 mL of refreshed culture medium, where nitrogen was briefly bubbled to lower O_2 saturation and thus prevent potential inhibitory effects on photosynthesis caused by oxygen supersaturation. Algae were kept in constant motion inside the vial using a magnetic stirrer. Water temperature was kept constant at 22 °C. For each replicate curve ($n=5$), 8 light levels were applied sequentially, increasing from 9 to 158 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Different irradiance levels were achieved by setting neutral density filters between the light source (W light quality) and the reaction chamber. Each light level was imposed for 10 min, except at 9 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, where exposure times was 15 min.

Changes in dissolved oxygen concentration were measured using a Microx4 fiber optic oxygen meter (PreSens, Germany). Net photosynthesis rates (NP; $\mu\text{mol O}_2 \text{ g FW}^{-1} \text{ h}^{-1}$) were calculated as follows:

$$NP = \frac{(O_2)_f - (O_2)_i}{T} \times \frac{V}{FW}$$

where $(O_2)_f$ = Final O_2 concentration ($\mu\text{mol L}^{-1}$); $(O_2)_i$ = Initial O_2 concentration ($\mu\text{mol L}^{-1}$); T = Incubation time (h); V = Volume of the chamber (L); FW = fresh weight (g).

PI curves were fitted with the model equation of Smith and Talling (Smith 1936; Talling 1955):

$$P = P_m \left[\frac{\alpha E}{(P_m^2 + (\alpha E)^2)^{1/2}} \right]$$

in which E is the irradiance, α is the ascending slope at limiting irradiances, and P_m is the maximum photosynthetic rate. Curves were fitted iteratively using SigmaPlot 11.0 (Systat Software, Inc.) and the parameters P_m and α as well as their standard error were estimated for a confidence interval of 95%. The minimum saturation irradiance, I_k , was calculated as the ratio between the estimated P_m and α for each treatment, incorporating error propagation.

Bromoform content

The bromoform content was determined by GC–MS analysis according to Romanazzi et al. (2021) and adapted to the amount of biomass available. Briefly, samples ($n=5$) from each treatment were split into three analytical replicates of 20 mg of powdered freeze-dried algae. These 20 mg were extracted twice with 10 mL of methanol sonicating at 0 °C for 30 min. Each extract

was centrifuged ($3000 \times g$ for 10 min at $4\text{ }^{\circ}\text{C}$) prior to the collection of the supernatant. The two extracts were combined and further diluted (0.5 mL of the mixture to 10 mL with methanol) before GC–MS analysis. Analysis was performed on a Bruker 456 TQ GC–MS gas chromatograph equipped with a triple quadrupole mass analyzer, and GC was fitted with a ZB-waxplus capillary column ($30\text{ m} \times 0.25\text{ mm i.d.}, 0.25\text{ }\mu\text{m}$, Phenomenex). The injector was maintained at $180\text{ }^{\circ}\text{C}$, operated in splitless mode and an injection volume of $1\text{ }\mu\text{L}$. The GC oven temperature was programmed to be held at $40\text{ }^{\circ}\text{C}$ for 1 min, raised at $16\text{ }^{\circ}\text{C min}^{-1}$ to $250\text{ }^{\circ}\text{C}$, and then held at $250\text{ }^{\circ}\text{C}$ for 2 min. Helium was used as the carrier gas, with a flow rate of 1 mL min^{-1} . MS Data Review software 8.2.1 (MS Workstation, Bruker Daltonics) was used for data analysis. A calibration standard curve for quantitation of bromoform was generated by serial dilution of a bromoform commercial standard (Merck Life Science S.L.). The concentration of bromoform was normalised according to the amount of dry biomass.

Data analysis

All results are presented as mean values \pm standard deviation of replicate samples ($n=5$), except when noted differently. To meet the analysis of variance (ANOVA) criteria of equal variance, data were square root-transformed. Differences in daily growth rates and bromoform concentration were tested using two-way ANOVAs, with light treatments (quality or intensity) and biomass density as the independent factors. Differences in the photosynthetic parameters observed at different light intensity treatments were tested using one-way ANOVA for each biomass density. Post hoc, multiple comparison analyses were done using the Turkey test. Differences were considered significant at a level of $p < 0.05$. Statistical analyses were performed using SigmaPlot 11.0 (Systat Software, Inc.).

Results

Effect of light quality

The DGR of *A. taxiformis* cultivated under different light qualities at two biomass density conditions is shown in Fig. 2. Average DGRs varied from 1.88 ± 0.16 to $8.08 \pm 0.49\%$ day^{-1} during the two weeks following the acclimation period. DGR was significantly affected by both light quality ($F_{3,32} = 138.557, p < 0.001$) and by biomass density ($F_{1,32} = 2012.773, p < 0.001$), with a significant interaction between both factors ($F_{3,32} = 5.660, p = 0.003$). DGRs decreased significantly (up to 2.8-fold) in cultures

stocked at high biomass density (2 g FW L^{-1}), regardless of the light quality used. The significantly highest DGRs were found at the High-BGR and W treatments at 0.5 g FW L^{-1} (8.08 ± 0.49 and $7.99 \pm 0.35\%$ day^{-1} , respectively). These two light qualities (High-BGR and W) also promoted the highest rates within density 2 g FW L^{-1} . There were no significant differences observed in DGRs between W and High-BGR, nor between BR and High-BR light qualities, regardless of the culture density (Fig. 2).

Regarding the concentration of bromoform in *A. taxiformis*, values ranged from 7.75 ± 2.63 to $18.56 \pm 5.11\text{ mg g}^{-1}\text{ DW}$ at the different light qualities and biomass densities (Fig. 3). Bromoform concentration was significantly affected by both

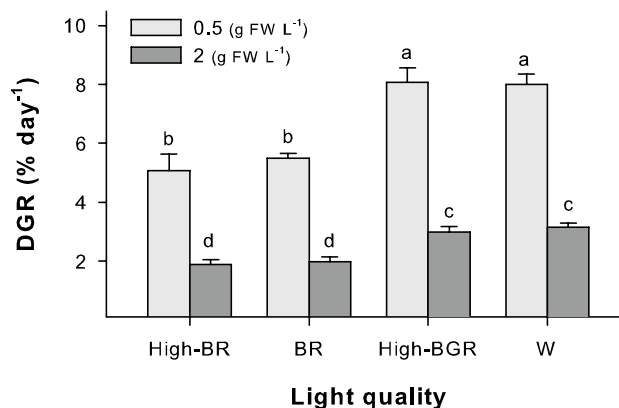


Fig. 2 Daily growth rates (DGR) of *Asparagopsis taxiformis* at different light qualities and biomass density conditions. Data are presented as mean \pm SD ($n=5$). Different letters represent significant differences among the treatments ($p < 0.05$). High-BGR=High Blue:Green:Red; High-BR=High Blue:Red; BR=Medium Blue:Red; and W=White

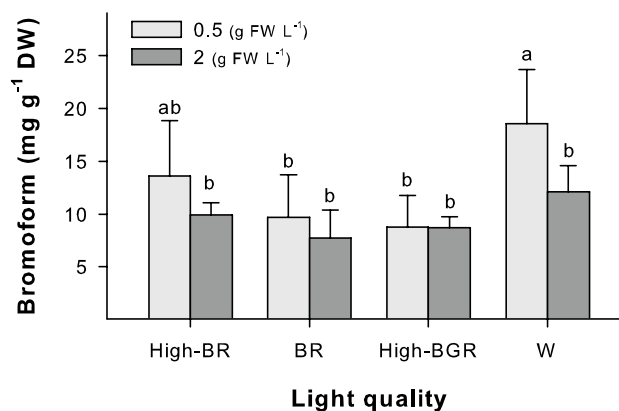


Fig. 3 Bromoform concentration ($\text{mg g}^{-1}\text{ DW}$) of *Asparagopsis taxiformis* cultivated at different light qualities and biomass density conditions. Data are presented as mean \pm SD ($n=5$). Different letters represent significant differences among the treatments ($p < 0.05$). High-BGR=High Blue:Green:Red; High-BR=High Blue:Red; BR=Medium Blue:Red; and W=White

light quality ($F_{3,32}=8.312$, $p<0.001$) and biomass density ($F_{1,32}=7.720$, $p=0.009$), and the interaction between these factors was not significant ($F_{1,32}=1.571$, $p=0.216$). The highest concentration of bromoform was in the combination of W light and 0.5 g FW L^{-1} ($18.56 \pm 5.11 \text{ mg g}^{-1} \text{ DW}$). Regardless of culture density, there were no significant differences in the concentration of bromoform among the High-BGR, BR and High-BR groups (Fig. 3).

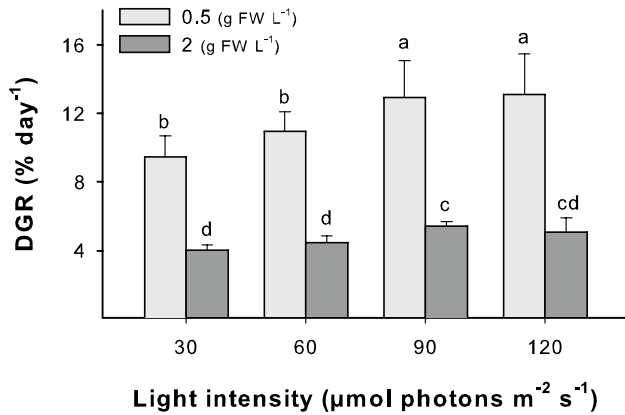
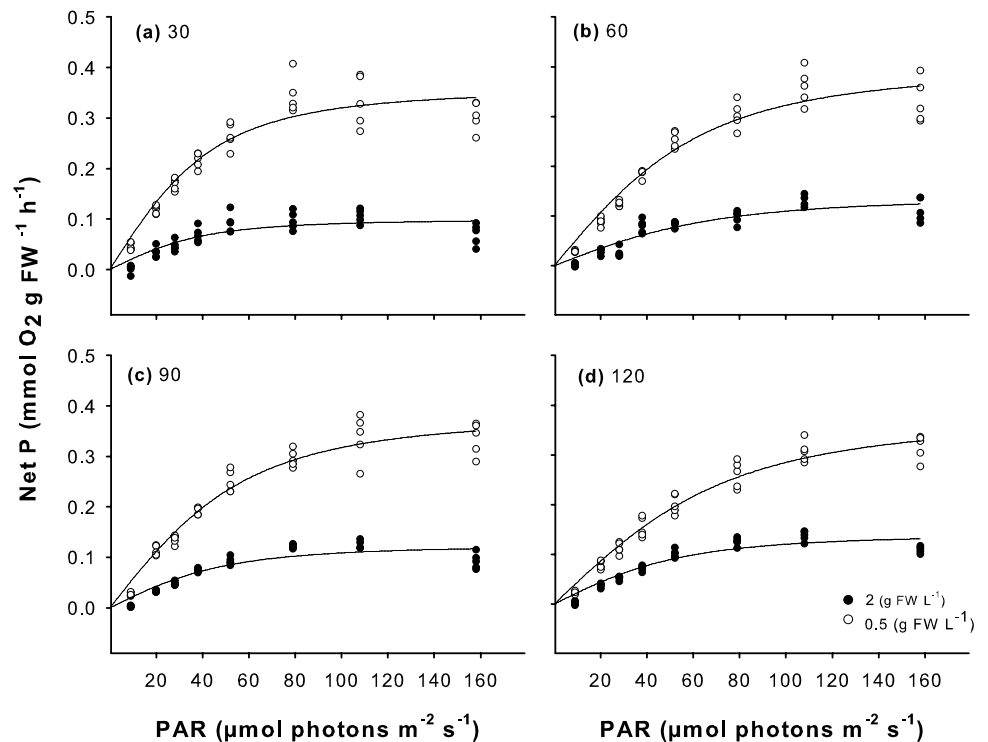


Fig. 4 Daily growth rates (DGR) of *Asparagopsis taxiformis* cultivated at different white light intensities and biomass density conditions. Data are presented as mean \pm SD ($n=5$). Different letters represent significant differences among the treatments ($p<0.05$)

Fig. 5 Light response curves of *Asparagopsis taxiformis* cultivated at different white light intensities (30 (a); 60 (b); 90 (c); and 120 (d) $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and two biomass density conditions. The model equation of Smith and Talling (Smith 1936; Talling 1955) was adjusted to the observed points



Effect of light intensity

The DGR of *A. taxiformis* cultivated under different W light intensities and biomass densities varied from 4.04 ± 0.29 to $13.10 \pm 2.35\% \text{ day}^{-1}$ during the two weeks following the acclimation period (Fig. 4). DGR differed significantly between light intensities ($F_{3,32}=28.462$, $p<0.001$) and biomass densities ($F_{1,32}=1089.073$, $p<0.001$) and there was no significant interaction between these two factors ($F_{1,32}=2.885$, $p=0.051$). Again, DGRs were significantly lower (up to 2.7-fold) for replicates cultivated at higher density (2 g FW L^{-1}), regardless of the light intensity used. The significantly highest DGRs were found at 90 and $120 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ treatments at 0.5 g FW L^{-1} (12.93 ± 2.14 and $13.10 \pm 2.35\% \text{ day}^{-1}$, respectively) and there was no significant difference between the two rates at these conditions. There were also no significant differences when *A. taxiformis* was cultivated under 30 or $60 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ within each biomass density level (Fig. 4).

The photosynthetic activity of *A. taxiformis* responded to increasing light intensity stimulation with a typical P-I hyperbolic-shaped response (Fig. 5). The parameter estimates, that describe the significant features of each P-I curve, are shown in Table 1. At the 0.5 g FW L^{-1} treatments, the maximum photosynthetic rates (P_m) were not affected by light intensity levels ($F_{3,16}=1.324$, $p=0.301$), while the photosynthetic quantum efficiency (α) decreased, and minimum saturation irradiance (I_k) increased with the increased light intensity levels ($F_{3,16}=11.312$, $p<0.001$ and $F_{3,16}=10.362$, $p<0.001$,

Table 1 Photosynthetic parameters of *Asparagopsis taxiformis* obtained after fitting the data with the Smith and Talling (Smith 1936; Talling 1955) P-I model

	Light treatment ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)			
	30	60	90	120
0.5 g FW L ⁻¹				
P_m	0.35 ± 0.01	0.39 ± 0.02	0.38 ± 0.01	0.37 ± 0.01
α	0.0072 ^a ± 0.0005	0.0055 ^{bc} ± 0.0003	0.0058 ^b ± 0.0003	0.0044 ^c ± 0.0002
I_k	49.65 ^a ± 3.91	72.13 ^{bc} ± 3.92	65.33 ^{ab} ± 4.01	84.39 ^c ± 5.01
r^2	0.93	0.95	0.96	0.97
2 g FW L ⁻¹				
P_m	0.10 ^a ± 0.01	0.14 ^b ± 0.01	0.12 ^{ab} ± 0.01	0.12 ^{ab} ± 0.01
α	0.0022 ± 0.0003	0.0018 ± 0.0002	0.0023 ± 0.0002	0.0020 ± 0.0002
I_k	45.41 ± 7.11	76.72 ± 10.72	53.96 ± 5.69	58.65 ± 6.91
r^2	0.78	0.86	0.88	0.92

P_m is the maximum photosynthetic rate ($\text{mmol O}_2 \text{ g FW}^{-1} \text{ h}^{-1}$), α is the ascending slope of the light-limited region of the response curves ($\text{mmol O}_2 \text{ g FW}^{-1} \text{ h}^{-1} (\mu\text{mol photons m}^{-2} \text{ s}^{-1})^{-1}$), I_k is the minimum saturation irradiance ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and r^2 is the coefficient of determination of the model adjustment to the data. Different letters indicate significant differences among treatments within each biomass density. Values are means ± se ($n=5$, $p < 0.001$)

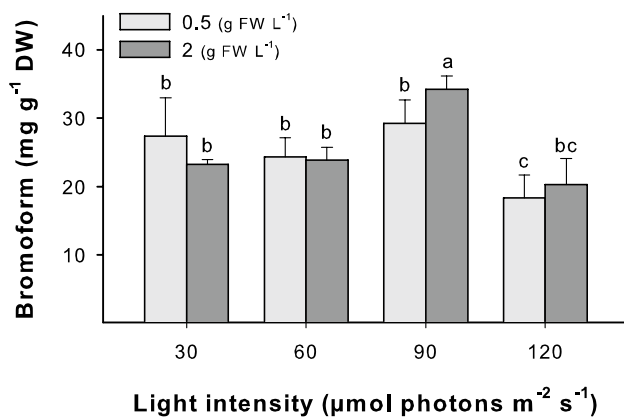


Fig. 6 Bromoform concentration ($\text{mg g}^{-1} \text{ DW}$) in *Asparagopsis taxiformis* cultivated under different white light intensities and biomass density conditions. Data are presented as mean ± SD ($n=5$). Different letters represent significant differences among the treatments ($p < 0.05$)

respectively). On the other hand, the 2 g FW L⁻¹ treatments, P_m rates were affected by light intensity levels ($F_{3,16} = 3.312$, $p = 0.0471$), presenting a trend of increasing at 60 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and then decreasing when the light intensity levels increased. These P_m were always lower (threefold or more) than those in treatments of 0.5 g FW L⁻¹. No significant difference was observed among α or I_k ($F_{3,16} = 0.937$, $p = 0.446$ and $F_{3,16} = 2.844$, $p = 0.071$, respectively).

Concerning the concentration of bromoform in *A. taxiformis*, values varied between 18.34 ± 3.32 and $29.26 \pm 3.45 \text{ mg g}^{-1} \text{ DW}$ at the different light intensities and biomass densities (Fig. 6). Bromoform concentration was significantly affected by light intensity ($F_{3,32} = 24.648$, $p < 0.001$) and not by biomass density levels tested ($F_{1,32} = 0.311$, $p = 0.581$), with a significant interaction

between both factors ($F_{3,32} = 3.404$, $p = 0.029$). The significantly highest concentration of bromoform was in the combination of 90 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ irradiance and 2 g FW L⁻¹ ($34.18 \pm 2.00 \text{ mg g}^{-1} \text{ DW}$). No significant differences were observed in the bromoform concentration between 30 and 60 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ treatments, regardless of biomass density, and bromoform concentration significantly decreased when *A. taxiformis* was cultivated under 120 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ at 0.5 g FW L⁻¹ (Fig. 6).

Discussion

Our results show that the light spectra containing intermediate wavelengths promoted the highest growth rates of *A. taxiformis*, compared to the other spectral conditions tested. Increases in the growth rate of up to 50% were observed in the W and High-BGR quality treatments at both biomass densities. The W and High-BGR treatments consisted of continuous wavebands containing a broad range of intermediate wavelengths (485–630 nm), whereas the lower performing BR and High-BR qualities consisted mostly of restricted blue and red lights. In this regard, it is known that Rhodophytes contain high phycoerythrin levels, a pigment that absorbs efficiently in the green region of the spectrum (495–570 nm) (Hurd et al. 2014). Therefore, the positive effect found on growth under intermediate wavelengths may be associated with a higher quantum efficiency under green-including light. The use of green and white lights also shown to promote the growth of other red seaweed species, such as *Halymenia floresii* (Godínez-Ortega et al. 2008) and *Pyropia haitanensis* (Wang et al. 2020). Moreover, green light was able to enhance the photosynthetic activity of *Meristotheca papulosa* (Borlongan et al. 2020), while in *Gracilaria gracilis* the electron transport

rate significantly increased under green light when compared to the control group (Ghedifa et al. 2021).

Asparagopsis taxiformis growth rates were lower under BR and High-BR supplemented light, and it should be noted that they were not affected by the different ratios of blue and red light. These results contrast those reported by Monro and Poore (2005), who found that blue light-enriched treatments induced faster growth rates in the congeneric species *A. armata*, and that a low ratio of blue to red light was correlated with lower growth rates. Two factors may have contributed to this discrepancy; firstly, a potential species-specific physiological difference between the two taxa (*A. armata* and *A. taxiformis*), and secondly the drastic difference between the dynamic light environment used in this study (tumble culture) compared to the stable light environment used in Monro and Poore's study (static culture). In this sense, the seaweed in the tumble culture may act as a non-neutral density filter, adding a degree of self-shading and preventing the wavelengths reflected or absorbed by the algae from penetrating deeper into the culture unit. As a result, the conditions of light quantity and quality that the algae are exposed to as they move up and down in the tumble culture vary significantly with their distance to the light source and may also vary with increasing biomass density, as higher densities increase the self-shading phenomenon. However, our findings suggest a weak interaction between light quality and biomass density on growth rates, as both biomass densities tested showed the same effects of light quality on growth. Hence, within the tested range of biomass densities, the growth-stimulating effect of intermediate wavelengths on *A. taxiformis* does not seem to be affected by the increasingly dynamic light environment associated with increased biomass density.

The highest bromoform concentration was obtained in the W light treatment, but only at density of 0.5 g FW L⁻¹. In seaweeds, bromoform, and other halogenated molecules are produced along with several reactive oxygen species that convert hydrogen peroxide into halogenated chemicals (Thapa et al. 2020). Our results suggest that for *A. taxiformis*, W light with low self-shading (lower biomass density) may induce some level of oxidative state inside the cells, leading to increasing bromoform production, compared to other light qualities. Zepeda et al. (2020) found an increase in antioxidant activity (an indicator of reactive oxygen species increases and eventual oxidative stress) under white light and white + blue light in *Solieria filiformis* and *Gracilaria cornea*, respectively. On the other hand, at the higher density of 2 g FW L⁻¹, the bromoform concentration in *A. taxiformis* was not sensitive to the different light qualities, suggesting that with this higher level of self-shading the W light does not induce enough of an oxidative state to result in higher bromoform levels. Thus, unlike growth, the stimulatory effect of W light on bromoform production appears to be counterbalanced by biomass increase.

Considering the above-mentioned results, the W light spectrum was selected to investigate the effects of light intensity on the growth rates and bromoform content of *A. taxiformis*. Growth responded positively to increasing light intensity regardless of the stocking biomass density used, with a plateau above 90 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, suggesting that, beyond this irradiance, increased light intensity does not promote increased growth. The most notable differences in growth were observed between the 2 stocking densities, regardless of the light intensity. For example, the seaweed stocked at 0.5 g FW L⁻¹ under the lowest light intensity of 30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ had a twofold higher growth than those cultures with the highest light intensity of 120 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ but stocked at 2 g FW L⁻¹ (higher self-shading). These results highlight the importance of adjusting biomass density (self-shading) in *A. taxiformis* tetrasporophyte cultures in order to manage biomass growth rates.

The parameters estimated of the P-I curves performed at the end of the experiment also highlight the power of the biomass density effect, where the maximum photosynthetic rates (P_m) and α values in the low biomass density were more than 3.5-fold higher than in the 2 g FW L⁻¹ cultures, possibly due to the decrease in the available light associated with self-shading, whereas changing light intensity within the same biomass density had mild effects on these two parameters. At 0.5 g FW L⁻¹, increasing light intensities above 30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ did not induce significant alterations in the P_m , suggesting that *A. taxiformis* is capable of optimal photosynthetic efficiency at low light intensities. This is corroborated by the significantly higher initial slope (α) of the P-I curve at 30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. These photosynthetic parameters suggests that *A. taxiformis* is a shade-type macroalgae (Reiskind et al. 1989), as also concluded previously by Mata et al. (2006) for the tetrasporophyte of *A. armata*. At the culture density of 2 g FW L⁻¹, P_m increased from 30 to 60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and then tended to decrease at the uppermost irradiance levels, and no changes were observed in α . The saturating irradiance (I_k) parameter provides valuable information to set the optimal irradiance for the cultures. Seaweed should not be continuously exposed to the saturating irradiance levels (I_k) as it may cause damage to the photosynthetic apparatus. In this sense, our results show that the cultures at the highest light intensity treatments (90 and 120 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) were already above the saturation irradiance, for both culture densities (for example, at 90 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, I_k was 65.2 and 53.9 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at densities of 0.5 and 2 g FW L⁻¹, respectively). This result suggests that long-term cultures of *A. taxiformis* should be maintained at light intensities no higher than 60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. It should be noted that all the cultures at light intensities above 60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ had filamentous cyanobacteria developing amidst the branches of *A. taxiformis* after 21 days of cultivation, while at 30 and 60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ no contamination was noticeable.

Contamination control in cultures of *Asparagopsis* is a significant challenge (Dishon et al. 2023), due to the size and filamentous nature of *Asparagopsis* tetrasporophytes. As such, maintaining *A. taxiformis* cultures at irradiances between 30 and 60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ is advisable in order to prevent the development of contamination, with the caveat of a sub-optimal growth rates.

There was no evident pattern between bromoform content, irradiance levels and biomass density. Notably, the biomass subjected to the highest light exposure (higher irradiance of 120 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ and lower self-shading at 0.5 g FW L^{-1}) had the lowest bromoform content. This goes against the initial expectations, that increasing irradiances that lead to hydrogen peroxide production and oxidative stress (Dummermuth et al. 2003) result in increasing bromoform synthesis (Ohsawa et al. 2001). However, it is in accordance with the results obtained by Mata et al. (2011) for *A. taxiformis*, where exposure to oxidative stress, through the addition of hydrogen peroxide in the culture water, inhibited the activity of iodoperoxidase (used as a proxy for bromoperoxidase, the enzyme involved in the production of bromoform) and reduced the bromoform concentration over exposure time. After 8 days of exposure to hydrogen peroxide the bromoform levels were 70% lower than initial values (Mata et al. 2011). In the current study, *A. taxiformis* was exposed to potentially oxidative stress inducing irradiances at 120 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (above the I_k) for 21 days, which may have inhibited the synthesis of bromoform. This pattern was similar in the marine diatom *Ditylum brightwellii* (Abe et al. 2022), where exposure to irradiances of 120 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ tended to reduce the relative production rates of bromoform, but stimulated the production of other halogenated compounds, such as chloroform and dibromomethane. A possible explanation for the decrease of bromoform content with increasing light (and hydrogen peroxide) could reside in the prosthetic group of the haloperoxidase. Two distinct types of marine haloperoxidases are identified in algae: those that contain vanadium as a cofactor and those containing Fe-heme (Butler and Walker 1993). Heme-containing peroxidases are inhibited at much lower concentration of hydrogen peroxide than vanadium-enzyme activity (Zuurbier et al. 1990). Thapa et al (2020), recently demonstrated that vanadium-dependent haloperoxidases are involved in bromoform synthesis but also detected multiple highly transcribed heme-dependent peroxidases in the *Asparagopsis* transcriptomes. The heme type haloperoxidase of the unicellular rhodophyte *Porphyridium purpureum*, was inhibited at relatively low concentrations of hydrogen peroxide concentrations (Murphy et al. 2000), and when exposed to high irradiance did not result in increased production of halogenated compounds (Scarratt and Moore 1999).

Our study shows that light containing intermediate wavelengths supports fast-growing *A. taxiformis* tetrasporophytes in indoor cultures, with white light being the preferred option,

as it also promotes higher bromoform content. Additionally, it is suggested that cultures stocked with up to 2 g FW L^{-1} of biomass should be maintained below the irradiance of 60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ to prevent photosynthetic stress and to avoid the development of biological contamination over time in culture, even if higher growth rates could be achieved with higher irradiances. These results provide valuable information to optimise the production of biomass and bioactive compounds in indoor cultures of the genus *Asparagopsis*.

Authors' contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Raquel Torres, Ana Campos, and Jacob Goldman. The first draft of the manuscript was written by Raquel Torres and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding Open access funding provided by FCTIFCCN (b-on). This study was financially supported by Greener Grazing, LLC, and by Portuguese national funds from FCT—Foundation for Science and Technology, through projects UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020.

Data availability The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Conflicts of interest The authors declare no competing interests.

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References

- Abe M, Okuda Y, Hashimoto S (2022) Effects of light intensity on the production of VSLs from the marine diatom *Ditylum brightwellii*. *J Atmos Chem* 79:1–6
- Andreakis N, Costello P, Zanolla M, Saunders GW, Mata L (2016) Endemic or introduced? Phylogeography of *Asparagopsis* (Florideophyceae) in Australia reveals multiple introductions and a new mitochondrial lineage. *J Phycol* 52:141–147
- Borlongan IA, Suzuki S, Nishihara GN, Kozono J, Terada R (2020) Effects of light quality and temperature on the photosynthesis and pigment content of a subtidal edible red alga *Meristotheca papulosa* (Solieriaceae, Gigartinales) from Japan. *J Appl Phycol* 32:1329–1340

- Butler A, Walker J (1993) Marine haloperoxidases. *Chem Rev* 93:1937–1944
- Dishon G, Resetaritis HM, Tsai B, Black K, Grossmann J, Smith JE (2023) Image-based analysis and quantification of biofouling in cultures of the red alga *Asparagopsis taxiformis*. *J Appl Phycol* 35:209–218
- Dummermuth AL, Karsten U, Fisch KM, König GM, Wiencke C (2003) Responses of marine macroalgae to hydrogen-peroxide stress. *J Exp Mar Biol Ecol* 289:103–121
- FAO (2020) Global Forest Resources Assessment 2020: Main Report. FAO, Rome
- Ghedifa AB, Vega J, Korbee N, Mensi F, Figueroa FL, Sadok S (2021) Effects of light quality on the photosynthetic activity and biochemical composition of *Gracilaria gracilis* (Rhodophyta). *J Appl Phycol* 33:3413–3425
- Godínez-Ortega JL, Snoeijs P, Robledo D, Freile-Pelegri Y, Pedersén M (2008) Growth and pigment composition in the red alga *Halymenia floresii* cultured under different light qualities. *J Appl Phycol* 20:253–260
- Greff S, Zubia M, Genta-Jouve G, Massi L, Perez T, Thomas OP (2014) Mahorones, highly brominated cyclopentenones from the red alga *Asparagopsis taxiformis*. *J Nat Prod* 77:1150–1155
- Guillard RRL (1975) Culture of phytoplankton for feeding marine invertebrates. In: Smith WL, Chanley MH (eds) *Culture of Marine Invertebrate Animals: Proceedings — 1st Conference on Culture of Marine Invertebrate Animals* Greenport. Plenum Press, NY, pp 29–60
- Hurd CL, Harrison PJ, Bischof K, Lobban CS (2014) *Seaweed ecology and physiology*. Cambridge University Press
- Hutson KS, Mata L, Paul NA, de Nys R (2012) Seaweed extracts as a natural control against the monogenean ectoparasite, *Neobenedenia sp.*, infecting farmed barramundi (*Lates calcarifer*). *Int J Parasitol* 42:1135–1141
- Kinley RD, de Nys R, Vucko MJ, Machado L, Tomkins NW (2016) The red macroalgae *Asparagopsis taxiformis* is a potent natural antimethanogenic that reduces methane production during in vitro fermentation with rumen fluid. *Anim Prod Sci* 56:282–289
- Kinley RD, Martinez-Fernandez G, Matthews MK, Nys RD, Magnusson M, Tomkins NW (2020) Mitigating the carbon footprint and improving productivity of ruminant livestock agriculture using a red seaweed. *J Clean Prod* 259:120836
- Liang Z, Liu F, Wang W, Zhang P, Yuan Y, Liu Y, Yao H, Jia R, Sun X, Wang F (2022) Physiological and biochemical responses to light and temperature stress in free-living conchocelis of *Neopyropia katadae* (Bangiales, Rhodophyta). *J Appl Phycol* 34:1059–1072
- Machado L, Magnusson M, Paul NA, Kinley R, Nys RD, Tomkins N (2016) Identification of bioactives from the red seaweed *Asparagopsis taxiformis* that promote antimethanogenic activity in vitro. *J Appl Phycol* 28:3117–3126
- Mata L, Gaspar H, Justino F, Santos R (2011) Effects of hydrogen peroxide on the content of major volatile halogenated compounds in the red alga *Asparagopsis taxiformis* (Bonnemaisoniaceae). *J Appl Phycol* 23:827–832
- Mata L, Schuenhoff A, Santos R (2010) A direct comparison of the performance of the seaweed biofilters, *Asparagopsis armata* and *Ulva rigida*. *J Appl Phycol* 22:639–644
- Mata L, Silva J, Schuenhoff A, Santos R (2006) The effects of light and temperature on the photosynthesis of the *Asparagopsis armata* tetrasporophyte (*Falkenbergia rufolanosa*), cultivated in tanks. *Aquaculture* 252:12–19
- Mata L, Wright E, Owens L, Paul N, Nys RD (2013) Water-soluble natural products from seaweed have limited potential in controlling bacterial pathogens in fish aquaculture. *J Appl Phycol* 25:1963–1973
- Monro K, Poore AGB (2005) Light quantity and quality induce shade-avoiding plasticity in a marine macroalga. *J Evol Biol* 18:426–435
- Murphy C, Moore R, White R (2000) Peroxidases from marine microalgae. *J Appl Phycol* 12:507–513
- Nilsson J, Martin M (2022) Exploratory environmental assessment of large-scale cultivation of seaweed used to reduce enteric methane emissions. *Sustain Prod Consumpt* 30:413–423
- Ohsawa N, Ogata Y, Okada N, Itoh N (2001) Physiological function of bromoperoxidase in the red marine alga, *Corallina pilulifera*: production of bromoform as an allelochemical and the simultaneous elimination of hydrogen peroxide. *Phytochemistry* 58:683–692
- Paul NA, de Nys R, Steinberg PD (2006) Chemical defence against bacteria in the red alga *Asparagopsis armata*: Linking structure with function. *Mar Ecol Prog Ser* 306:87–101
- Pinteus S, Lemos MFL, Alves C, Neugebauer A, Silva J, Thomas OP, Botana LM, Gaspar H, Pedrosa R (2018) Marine invasive macroalgae: Turning a real threat into a major opportunity - the biotechnological potential of *Sargassum muticum* and *Asparagopsis armata*. *Algal Res* 34:217–234
- Reiskind JB, Beer S, Bowes G (1989) Photosynthesis, photorespiration and ecophysiological interactions in marine macroalgae. *Aquat Bot* 34:131–152
- Romanazzi D, Sanchez-Garcia C, Svenson J, Mata L, Pes K, Hayman CM, Wheeler TT, Magnusson M (2021) Rapid analytical method for the quantification of bromoform in the red seaweeds *Asparagopsis armata* and *Asparagopsis taxiformis* using gas chromatography-mass spectrometry. *ACS Agric Sci Technol* 1:436–442
- Roque BM, Salwen JK, Kinley R, Kebreab E (2019) Inclusion of *Asparagopsis armata* in lactating dairy cows' diet reduces enteric methane emission by over 50 percent. *J Clean Prod* 234:132–138
- Roque BM, Venegas M, Kinley RD, Nys RD, Duarte TL, Yang X, Kebreab E (2021) Red seaweed (*Asparagopsis taxiformis*) supplementation reduces enteric methane by over 80 percent in beef steers. *PLoS One* 16:e0247820
- Scarratt MG, Moore RM (1999) Production of chlorinated hydrocarbons and methyl iodide by the red microalga *Porphyridium purpureum*. *Limnol Oceanogr* 44:703–707
- Schuenhoff A, Mata L, Santos R (2006) The tetrasporophyte of *Asparagopsis armata* as a novel seaweed biofilter. *Aquaculture* 252:3–11
- Smith EL (1936) Photosynthesis in relation to light and carbon dioxide. *Proc Natl Acad Sci U S A* 22:504–511
- Talling JF (1955) The relative growth rates of three plankton diatoms in relation to underwater radiation and temperature. *Ann Bot* 19:329–341
- Thapa HR, Lin Z, Yi D, Smith JE, Schmidt EW, Agarwal V (2020) Genetic and biochemical reconstitution of bromoform biosynthesis in *Asparagopsis* lends insights into seaweed reactive oxygen species enzymology. *ACS Chem Biol* 15:1662–1670
- Torres R, Mata L, Santos R, Alexandre A (2021) Nitrogen uptake kinetics of an enteric methane inhibitor, the red seaweed *Asparagopsis armata*. *J Appl Phycol* 33:4001–4009
- Wang X, Zhang P, Wu Y, Zhang L (2020) Effect of light quality on growth, ultrastructure, pigments, and membrane lipids of *Pyropia haitanensis*. *J Appl Phycol* 32:4189–4197
- Yong YS, Yong WTL, Anton A (2013) Analysis of formulae for determination of seaweed growth rate. *J Appl Phycol* 25:1831–1834
- Zanolla M, Altamirano M, Carmona R, Rosa JDL, Sherwood A, Andreakis N (2015) Photosynthetic plasticity of the genus *Asparagopsis* (Bonnemaisoniales, Rhodophyta) in response to temperature: implications for invasiveness. *Biol Invasions* 17:1341–1353
- Zanolla M, Carmona R, Mata L, Rosa JDL, Sherwood A, Barranco CN, Muñoz AR, Altamirano M (2022) Concise review of the genus *Asparagopsis* Montagne, 1840. *J Appl Phycol* 34:1–17
- Zepeda E, Freile-Pelegri Y, Robledo D (2020) Nutraceutical assessment of *Solieria filiformis* and *Gracilaria cornea* (Rhodophyta) under light quality modulation in culture. *J Appl Phycol* 32:2363–2373

- Zuccarello G, Burger G, West J, King RJ (1999) A mitochondrial marker for red algal intraspecific relationships. *Mol Ecol* 8:1443–1447
- Zuurbier KW, Bakkenist AR, Wever R, Muijsers AO (1990) The chlorinating activity of human myeloperoxidase, high initial activity at neutral pH value and activation by electron donors. *Biochim Biophys Acta - Protein Struct Molec Enzymol* 1037:140–146

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