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OPTIMIZING THE GROWTH OF THE RED SEAWEED ASPARAGOPSIS TAXIFORMIS BY MANAGING LIGHT QUALITY AND INTENSITY

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Statement of authorship

I declare to be the author of this work which, is original and unpublished. Authors and works consulted are duly cited in the text and are included in the list of references.

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RESUMO

As macroalgas vermelhas do género Asparagopsis são produtores prolíficos de compostos naturais bioativos, em particular compostos halogenados com fortes propriedades antimicrobianas. O género compreende a espécie A. taxiformis, distribuída em águas quentes e tropicais, e A. armata, distribuída em águas frias a temperadas. Asparagopsis spp. exibem um ciclo de vida diploide trifásico. Estas algas marinhas têm aplicações promissoras como aditivo para rações com propriedades anti-metanogénicas em animais ruminantes. A cultura destas algas à escala comercial é um tema de crescente interesse e exigirá a capacidade de produzir altos rendimentos de biomassa para o que é necessário compreender as condições abióticas que promovem taxas de crescimento mais altas. O tetraesporófito de A. taxiformis foi selecionado para este estudo porque ser a fase do ciclo de vida mais resiliente e a espécie apresentar maior tolerância a temperaturas quentes, uma vantagem para o cultivo em grande escala, uma vez que a maioria da aquacultura de algas marinhas ocorre em águas quentes na Ásia. A luz é a fonte de energia utilizada na fotossíntese e, portanto, um fator essencial para o crescimento de todos os organismos fotossintéticos. A qualidade e a quantidade da luz são fatores importantes que podem ser manipulados para controlar o crescimento das algas. Diferentes bandas do espectro luminoso são capturadas com eficiência diferente pelos cloroplastos e esta resposta é específica da espécie e está relacionada com a composição dos pigmentos. Vários estudos demonstraram que diferentes proporções de componentes espectrais influenciam o crescimento de macroalgas. A quantidade de luz é crucial para o crescimento, pois a luz excessiva causa stress e danos celulares, enquanto a luz insuficiente afeta negativamente a taxa fotossintética das algas marinhas. Tanto a qualidade quanto a quantidade da luz podem influenciar o fenótipo por meio de fotomorfogenética, que pode levar a alterações na taxa de crescimento. A densidade da biomassa afeta o auto-sombreamento das algas desempenhando um papel crítico na regulação das condições de luz na cultura de algas marinhas. Este estudo baseia-se em conclusões anteriores sobre a resposta de Asparagopsis à quantidade e qualidade da luz em condições estáticas, avaliando esses fatores na cultura tipo bottom-aerated batch exchange tumble, um sistema em que as algas estão em constante movimento num ambiente de luz dinâmico que mais se aproxima das condições de cultivo usadas na cultura comercial de algas marinhas. Neste estudo pretendemos compreender os efeitos da qualidade e quantidade da luz em culturas de Asparagopsis taxiformis em diferentes densidades de biomassa, como forma de gerir as condições de luz para otimizar as taxas de crescimento. Este estudo avaliou (1) a resposta da

fotossíntese a diferentes níveis de irradiância; (2) os efeitos de diferentes qualidades espectrais no crescimento da biomassa; e (3) os efeitos de uma gama de intensidades de luz no crescimento. Todos os fatores foram testados em diferentes densidades de biomassa. As respostas fotossintéticas a vários níveis de irradiância foram avaliadas seguindo a produção de oxigénio a curto prazo, enquanto os efeitos da quantidade e qualidade de luz no crescimento foram avaliados durante um período de três semanas para níveis de densidade de biomassa. Os resultados indicam que os espectros que incluem comprimentos de onda intermediários promovem taxas de crescimento mais altas em ambos os níveis de densidade de biomassa testados. Não foram encontradas diferenças no crescimento entre algas cultivadas sob qualidades de luz com diferentes proporções de luz vermelha e azul. As taxas de crescimento aumentaram com o aumento da intensidade da luz dentro das faixas de irradiância testadas, independentemente das densidades de biomassa. No entanto, os resultados da experiência sobre os efeitos da quantidade de luz podem ter sido afetados por uma variável não controlada (confounding). Este trabalho pode ser a base para a futura investigação, dos efeitos no crescimento desta macroalga, de luz com uma maior proporção de comprimentos de onda intermediários. No geral, as taxas de crescimento observadas neste trabalho são comparáveis às de outras algas vermelhas e às reportadas para Asparagopsis. Investigações adicionais sobre os efeitos da intensidade da luz em Asparagopsis devem usar um processo de aclimatação das algas gradual, evitando o stress induzido por luminosidade intensa, que poderá influenciar os resultados. A qualidade da luz e, particularmente, a quantidade têm um efeito profundo no crescimento de A. taxiformis e podem ser manipuladas para aumentar a produção de biomassa em culturas de interior.

ABSTRACT

The red macroalgal genus *Asparagopsis* is a prolific producer of bioactive natural compounds with promising applications as a feed additive with anti-methanogenic properties in ruminant animals. Achieving large scale aquaculture is the subject of increasing interest and will require the ability to produce high biomass yields by understanding the culture and abiotic conditions that promote higher growth rates. In this study we aimed at understanding the effects of light quality and quantity in *Asparagopsis taxiformis* cultures at different biomass densities, as a way to managing light conditions to optimize growth rates. This study evaluated (1) the response of photosynthesis to different irradiance levels (PI curves); (2) the effects of different spectral qualities on biomass growth; and (3) the effects of a range of light intensities on growth, all at different biomass densities. We found that spectra which include intermediate wavelengths promoted higher growth rates and that growth rates increased with increasing light intensity within the range of irradiances tested and regardless of the biomass densities. Overall, light quality and particularly quantity have a profound effect on *A. taxiformis* growth and can be manipulated to increase the production of biomass in indoor cultures.

ABBREVIATIONS

Abbreviation	Meaning
ANOVA	analysis of variance
BL	blue light
BR	blue: red (experimental light quality used in this thesis, see Figure 1)
CCMAR	Centro de Ciências do Mar
CH_4	methane
CO_2	carbon dioxide
DGR	daily growth rate
DW	dry weight
FW	fresh weight
H_2	hydrogen
HighBGR	high blue: green: red (experimental light quality used in this thesis, see Figure 1)
HighBR	high blue: red (experimental light quality used in this thesis, see Figure 1)
Ik	saturation irradiance
LED	light emitting diode
O_2	oxygen
OM	organic matter
PAR	photosynthetically active radiation
PERMANOVA	permutational analysis of variance
PI	Photosynthesis-irradiance
P_{max}	maximum photosynthetic rate
RL	red light
W	white (experimental light quality used in this thesis, see Figure 1)
WL	white light
α	photosynthetic quantum efficiency at limiting irradiance

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1. INTRODUCTION

1.1. The *Asparagopsis* genus

Asparagopsis is a genus of saltwater macroalgae seaweed of the family Bonnemaisoniaceae (Rhodophyta). The genus comprises the cold-temperate distributed *A. armata* and the tropical to warm temperate distributed *A. taxiformis* (Guiry and Guiry, 2021). *Asparagopsis* spp. display a triphasic diplohaplontic life cycle. Haploid gametophytes are morphologically distinct from diploid tetrasporophytes, resulting in previous taxonomic misclassification as a unique species "*Falkenbergia rufolanosa*" (Feldmann and Feldmann, 1942).

Asparagopsis spp. produce a wide variety and high quantity of secondary metabolites, particularly halocarbons with potent and broad antimicrobial activity (Burreson et al., 1976; McConnell and Fenical, 1977) which act as chemical defenses against herbivores and microorganisms' settlement (Paul et al., 2006). Bromoform is the most prominent halocarbon in *Asparagopsis*, with a content as high as 3.1% of its dry weight (DW)), but usually making up a mean of 1.45% DW of the tetrasporophyte of *A. armata* and 1.67% DW of the gametophyte (Paul et al., 2006). Early research on the biotechnological potential of *Asparagopsis* focused on applications for the cosmetic and pharmaceutical industries (e.g., de Nys et al., 1995; Moigne, 1998; Haslin et al., 2001). More recently, interest in *Asparagopsis* seaweed has focused on applications as a feed additive, including for disease management and growth promotion in fish aquaculture (Reverter et al., 2014; Thépot et al., 2021) and as an anti-methanogenic for ruminant animals (e.g., Machado et al., 2014; Li et. al., 2016; Silwer, 2018). This latter application has led to strong interest in the mass production of *Asparagopsis* as a means to combat climate change.

1.2 *Asparagopsis* as an anti-methanogenic

Methane (CH_4) is a greenhouse gas with a global warming potential 28-fold higher than that of CO_2 (IPCC, 2014) and ruminant animal production is responsible for around 37% of anthropogenic CH_4 emissions, most of which are the result of enteric production (Kingston-Smith et al., 2010). Therefore, decreasing CH_4 emissions from the livestock sector is a major factor of reducing climate change. In the animal rumen, a symbiotic microbial community comprised of anaerobic fungi, anaerobic bacteria and protozoa ferment organic

matter (OM) to produce metabolic substrate molecules that provide energy to the animal, primarily as volatile fatty acids (Martin et al., 2010; Morgavi et al., 2010). Simultaneously, anaerobic fermentation end products hydrogen (H_2) and carbon dioxide (CO_2) are converted to CH_4 via a reduction pathway by methanogenic archaea (Morgavi et al., 2010). The anti-methanogenic activity of *Asparagopsis* is related to its remarkably high concentration of halocarbons, particularly of bromoform though other metabolites could contribute synergistically to its efficacy (Machado et al., 2016). Halocarbons act as a potent anti-methanogen by inhibiting a key Archaean enzymatic reaction (Wood et al., 1968; DiMarco et al., 1990). Chagas et al., (2019) assessed a variety of previously successfully demonstrated CH_4 mitigation strategies and found the treatment with *A. taxiformis* to be the most effective for CH_4 inhibition *in vitro*, with the lowest effect on rumen fermentation, with ca. 99% reduction of CH_4 production at inclusion rates of 1-2% (OM basis). Kinley et al., (2020) found that incorporating *A. taxiformis* in beef cattle feed at 0.20% (OM basis) resulted in 98% decrease of CH_4 production *in vivo*. Furthermore, the average daily weight gain of cattle was enhanced by 22% (Kinley et al., 2020). The energy requirement for CH_4 production in the rumen is estimated at 5- 15% of the gross energy in feed (Hristov et al., 2013; Van Nevel and Demeyer, 1996); thus, reducing methanogenesis may result in improved feed digestibility and conversion efficiency (Patra, 2012). However, achieving commercial scale production remains a major hurdle.

1.3. Cultivation of *Asparagopsis*

Seaweed aquaculture is a rapidly expanding sector. Biomass production rose from 13.5 million tonnes fresh weight (FW) in 2005 to 29.4 million tonnes FW in 2015 (FAO, 2018). Marine sea-based aquaculture in Asia comprises the vast majority of these numbers, with land-based aquaculture producing biomass for low-volume and high-value niche markets. *Asparagopsis* cultivation is still very limited with cultivation of *A. armata* gametophyte in the sea in Brittany, France (Kaas, 1998) and the sporophyte phase of *Asparagopsis* spp. on land in Algarve, Portugal (Mata, 2008). Meeting the needs of a high-volume market would require ocean-based cultivation and considering that almost all seaweed aquaculture occurs in warm waters in Asia (FAO, 2018), successful aquaculture of the warm water gametophyte of *A. taxiformis* is important. *A. taxiformis* aquaculture based on vegetative propagation of the gametophyte (as done with *A. armata*) is not feasible. To farm the gametophytes in the sea, a land-based indoor system is required for nursery production of seedlings. This allows the

manipulation of abiotic conditions to optimize the outcomes of the land-based stage, i.e., fast propagation of broodstock (sporophyte) biomass.

Light is a major factor affecting the growth of photosynthetic organisms. In indoor algae culture, artificial lighting is used to ensure biomass growth and if properly managed can greatly improve the cultivation performance. The management of light available to the seaweeds is done by controlling the spectral composition (light quality), the irradiance (intensity), and the algae biomass density in the culture vials. It is well established that these three factors have an impact on macroalgae growth (e.g., Dring, 1988; Figueroa et al., 1995; Talarico and Cortese, 1993; Demetropoulos and Langdon, 2004). Determining the effect of these variables on *A. taxiformis* would be an advantage to control growth and achieve high yields in indoor culture.

1.4. Light quality

Light spectral composition influences seaweed growth, metabolism, and vegetative development (Figueroa et al., 1995). Manipulation of light quality is a valuable tool in indoor macroalgae cultivation. The light quality most well suited for achieving high growth rates differs among species. Blue light (BL) has been shown to stimulate growth and regulate metabolic functions (Dring, 1988; Lobban and Harrison 2014), including cell division (Carrol et al., 1970) and enzyme synthesis (Roscher and Zetsche, 1986). Red light (RL) has also been linked to promoting growth and triggering physiological processes such as cell division, thylakoid stacking, regulating secondary growth patterns, and boosting carbon metabolism (Lobban and Harrison, 2014; Kreslavsky et al., 1997; Holdsworth, 1985; Figueroa et al., 1995). In an overview of red algae adaptive responses to light Talarico and Maranzana (2000) suggested that light qualities with differing variations of the ratios between spectral components may act as photomorphogenic ‘signals’ controlling algal metabolism and growth.

Macroalgae photosynthetic efficiency at a given wavelength is dependent on the pigment profile in the light absorption complex; we can relate an algae’s relative abundance of pigments with unique absorption spectra to the efficiency of capture of spectral components (Larkum, 2012). Light capture has been found to be negatively affected by short wavelengths in some species (e.g., blue; Figueroa et al., 1995). Quantum efficiency can decrease under lower wavelengths since more energy is contained per photon which can result in an increased portion of energy being dissipated as heat (Lüning and Dring, 1985).

Many experiments have demonstrated differing responses to various spectral distributions among macroalgae species, including rhodophytes. Carmona et al. (1996) demonstrated higher growth in *Gelidium sesquipedale* cultured under blue and red light compared to white light. In a study of the red seaweed *Halymenia floresii*, green wavelengths produced the highest growth rates, while red and blue played a role in inducing phycobiliprotein synthesis (Godínez-Ortega et al., 2008). Thien et al. (2016) demonstrated higher growth rates in *Kappaphycus alvarezi* in red light than in blue light. Recently, Zepeda et al. (2020) cultivated *Gracilaria cornea* and *Solieria filiformis* in five light quality treatments: white, blue, green, red and a combination of white and blue light over 3 weeks. In general, seaweeds subjected to the white and blue light treatment displayed significantly increased growth rate compared to the white, green, and red light treatments.

Monro and Poore (2005) showed that growth and morphology of the sporophyte of *A. armata* are plastic traits influenced by light quantity and quality. These authors demonstrated morphological variations in response to different light qualities, resulting in growth strategies characterized by the production of phalanx and guerrilla phenotypes. The phalanx phenotype was associated with higher second order branch production and growth while the guerrilla phenotype prioritizes resource allocation to branch elongation at the expense of new meristem production and growth. Thalli exposed to spectra with reduced ratios of B:R light exhibited branch elongation and slower growth. Far-red supplemented light yielded the most elongate phenotypes while medium to high ratios of B:R light optimized growth. These results suggest that high B:R light conditions could be used to promote compact phenotypes with greater second order branch production to optimize growth rates of *A. armata*. However, the results of Monro and Poore were obtained in static cultures with constant light conditions. In commercial aquaculture seaweeds are cultivated in 3-dimensional tanks with bottom aeration that keeps the seaweed in suspension to allow individual thali access to light at the surface of the culture vial (Mata et al., 2006). This results in self-shading and influences the light quality conditions in the culture. Determining the effects of light quality on *A. taxiformis* tetrasporophyte in tumble culture would contribute to the literature and advance efforts to develop cultivation of this species.

1.5. Light intensity

Light intensity is a vital factor affecting macroalgae growth with sub- and supra-optimal levels resulting in insufficient energy for photosynthesis and negative physiological effects including damaging the photosynthetic apparatus respectively (Lobban and Harrison,

2014). Light intensity is known to influence growth, metabolism, morphology, and pigment synthesis in red seaweed (reviewed in Talarico and Maranzana, 2000). Monro and Poore (2005) demonstrated that in *A. armata* tetrasporophyte higher irradiance levels (24-60 μmol photon m^{-2} s^{-1}) promoted growth, production of new meristems and second order branches, and was negatively correlated with branch lengths while lower irradiances (<24 μmol photon m^{-2} s^{-1}) produced longer branches and suppressed growth.

Understanding the photosynthetic response of an algae species to light intensity is an essential aspect of optimizing the light regime in cultivation. This can be done by performing photosynthesis-irradiance curves, more commonly referred as PI curves. The response is species specific and can be divided in three phases. The first phase of the curve corresponds to the positive response of photosynthesis to increasing irradiance until reaching the saturating irradiance (Ik), the slope during this phase is referred to as α. At the light saturated photosynthetic rate (P_{max}), other factors become limiting and photosynthetic rate plateaus with irradiance (second phase). The third phase is not always observable and corresponds to a decline in the photosynthetic rate at over-saturating irradiance (photoinhibition), that can be a dynamic process or, under specific conditions, become chronic and result in tissue damage (Aguirre-von-Wobeser et al., 2000). PI curves provide valuable data for algae culture, particularly to determine the saturating and photoinhibiting irradiances (μmol photon m^{-2} s^{-1}). Mata et al. (2006) showed that *Asparagopsis* sporophyte photosynthetic rates generally decline with intensities above 150 μmol m^{-2} s^{-1} with P_{max} of ~11 mg O_2 g DW^{-1} h^{-1} and α ~0.3 mg O_2 g DW^{-1} h^{-1} (μmol photon m^{-2} s^{-1})$^{-1}$. However, Zanolla et al. (2015) detected no reduction of photosynthetic rates as high as 600 μmol m^{-2} s^{-1}, with net photosynthetic rates reaching 3.5 mg O_2 g FW^{-1} h^{-1} and α 0.094 mg O_2 g FW^{-1} h^{-1} (μmol photon m^{-2} s^{-1})$^{-1}$. These PI curves were performed in single individuals with motionless biomass. They do not assess the effects of biomass density and do not capture all the information necessary to assess growth rates (e.g., photomorphogenetic effects). This data may or may not apply in tumble culture in which *A. taxiformis* is subject to a dynamic light environment. To determine how light quantity affects growth rate, multi-week trials in real culture conditions are needed, which allow for acclimation and to capture the effects of light conditions that take time to develop.

1.6. Algae density

In designing cultivation systems, it is important to consider not only the irradiance that produces the highest growth rates but also the specific culture conditions that control the

light energy reaching the algae. As Demetropoulos and Langdon (2004) showed, the stocking density is of particular importance in regulating the light quantity that algae are exposed to, because higher densities increase vegetative self-shading as individuals rotate within the tank. Since self-shading influences the light environment within the culture, biomass density may interact with the influence of light quality and quantity on growth.

1.7. Objectives

The current knowledge on the response of *Asparagopsis* to light quality and intensity in tumbling cultures is still limited and leaves unanswered questions. The effect of these factors on growth across biomass densities is important for efforts of scaling-up cultivation. The goal of this thesis was to determine how light can be managed to optimize the growth rates of *A. taxiformis* in indoor tumbling cultures at different biomass densities. More specifically we assessed the (1) response of photosynthesis to different irradiance levels (PI curves); (2) the effects of different spectral qualities on biomass growth; and (3) the effects of a range of light intensities on growth using the selected light quality.

2. MATERIALS AND METHODS

2.1. Algae, media, and culture conditions

We used a clonal *A. taxiformis* tetrasporophyte isolate from the culture collection of Dr. Leonardo Mata at the Centro de Ciências do Mar (CCMAR) in Faro, Portugal. The culture medium was comprised of UV-sterilized natural seawater (salinity of 33ppt and pH of 8.2) enriched with f/2 medium based on Guillard (1975) (see Table 1 for molar concentrations). Seaweeds were incubated in a FitoClima environmentally controlled growth chamber (Aralab, Portugal).

Table 1. Culture medium components and molar concentrations. F/2 seawater enrichment was prepared based on Guillard (1975).

	Component	µmol L^{-1} of seawater in final medium
Nitrate solution		
	$NaNO_3$	882
Phosphate solution		
	$NaH_2PO_4 \cdot H_2O$	36.2
Trace metal solution		
	$FeCl_3 \cdot 6H_2O$	11.7
	$Na_2EDTA \cdot 2H_2O$	11.7
	$MnCl_2 \cdot 4H_2O$	0.91
	$ZnSO_4 \cdot 7H_2O$	0.0765
	$Na2MoO_4 \cdot 2H_2O$	0.026
	$CoCl_2 \cdot 6H_2O$	0.042
	$CuSO_4 \cdot 5H_2O$	0.0393
Vitamin solution		
	Thiamine	0.296
	Biotin	0.00205
	Cyanocobalamin	0.000369

2.2. Photosynthesis-irradiance curves

Photosynthesis-irradiance (PI) curves of *A. taxiformis* tetrasporophyte were evaluated at four biomass densities (0.1, 0.5, 1.0, and 2.0 g FW L^{-1}) by measuring oxygen production in response to 12 irradiance levels, ranging from 5 to 215 µmol photon m^{-2} s^{-1}. Prior to measurement of photosynthesis, algae were acclimated in the FitoClima for 7 days with controlled light, temperature and photoperiod (30 µmol photon m^{-2} s^{-1}, 22º C and 12:12 h respectively), in 300 mL vials with culture medium (f/2 enriched UV-sterilized natural seawater, salinity 33ppt, pH 8.2), supplied with constant bottom aeration. For the PI curves, 30, 150, 300, or 600 mg FW of *A. taxiformis* were incubated in vials 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. The vials were exposed to each irradiance level for approximately 10 minutes each (except at 5 and 9 µmol photon m^{-2} s^{-1}, where exposure times were 20 and 15 minutes, respectively). Different irradiance levels were achieved using neutral density optical filters. Dissolved oxygen concentration was measured before and after exposure to each irradiance level using a Microx4 fiber optic oxygen meter (PreSens, Regensburg, Germany). Oxygen production was calculated as the difference in dissolved oxygen concentration before and after exposure to each irradiance. Photosynthetic rates were calculated as O_2 production per g FW of biomass per hour. PI curves for each biomass density were performed using 5 replicates (except for the 0.5 g FW L^{-1} biomass density level in which n=4). PI curves were fitted with the model equation of Platt et al. (1980):

$$P = P_{max} (1- \exp(-\alpha I/P_{max})) \exp(-\beta I/P_{max})$$

where P represents net photosynthetic rate (µmol O_2 g FW^{-1} h^{-1}), P_{max} is the maximum photosynthetic rate (µmol O_2 g FW^{-1} h^{-1}), I is irradiance (µmol photon m^{-2} s^{-1}), α is the photosynthetic quantum efficiency at limiting irradiance (µmol O_2 g FW^{-1} h^{-1}(µmol photon m^{-2} s^{-1}) $^{-1}$) and β is the photosynthetic decline coefficient at saturating irradiance. The Platt et al. model was selected because the model has been demonstrated to provide a good fit for *Asparagopsis* PI data (Mata et al. 2006). The SigmaPlot software package was used to fit the curves. Tests for significant differences in the photosynthetic parameters P_{max}, α, and saturation irradiance, Ik (µmol photon m^{-2} s^{-1}), observed at different biomass densities were performed using one-way analyses of variance (ANOVAs). Post hoc Tukey's tests were performed to compare significant differences in biomass density levels when significant differences were found ($p < 0.05$). The photosynthetic parameters obtained from the PI curves were used to inform the selection of light intensity used in the subsequent light quality experiment. In particular, the saturation irradiance was used to inform the selection of an irradiance within the light limited portion of the PI curve.

2.3. Light quality

This experiment utilized a 4 x 2 factorial design (light quality x biomass density) to test the effects of light quality on growth in high- and low-density *A. taxiformis* tetrasporophyte culture. Biomass density levels 0.5 and 2.0 g FW L^{-1} were used as these

levels approximately correspond to the low and high end of biomass density levels which allow for high growth in indoor production. The experiment used 4 light emitting diodes (LEDs) programmed to produce distinct relative spectral distributions with equivalent total photosynthetically active radiation (PAR) which are presented in Figure 1: medium blue:red (BR), high blue:red (highBR), high blue:green:red (highBGR), and White (W). Experimental vials were installed inside white foamboard cabinets to create homogenous and discrete light fields for all light sources. Light emitting diodes are expedient for algae cultivation and experiments examining the effects of wavelength because of their energy efficiency, low heat output and narrow emission spectra which enables testing of specific light qualities (Bourget, 2008). For the purposes of this study blue light was regarded as the waveband from 400-485 nm and red light from 630-700 nm while the intermediate waveband (486-629 nm) was referred to as green light, although this waveband also includes orange and yellow spectra. Irradiance was controlled in all light quality treatments at 30 μmol photon m^{-2} s^{-1} as this irradiance fell within the light limited portion of the PI curves observed at both 0.5 and 2.0 g FW L^{-1}. For the experiment, 0.25 or 1.0 g FW of *A. taxiformis* were placed in Erlenmeyer flasks with 500 mL of f/2 enriched UV-sterilized natural seawater (salinity 33ppt, pH 8.2) and incubated in the FitoClima with controlled temperature and photoperiod (22º C and 12:12 h respectively). Culture flasks were supplied with constant bottom aeration to provide water motion and supply carbon for growth. 5 replicates per treatment were used (n=40 in total). Algae were incubated for 3 weeks with the first week considered as the acclimatory period. At the end of each week algae were removed from the media, centrifuged (30 seconds, 3000 RPM) to separate the algae from any remaining media on the algae surface, weighed, and returned to refreshed media at the experimental stocking densities. The daily growth rate (DGR), expressed as % day^{-1}, was calculated using the formula:

$$DGR = [ln(N_2 /N_1)/(t_2-t_1)]*100,$$

where N_2 and N_1 represent fresh weights at times t_2 and t_1 respectively. Data analysis was conducted in the program R. Tests for significant differences in DGR at different factor levels and factor interaction were performed using permutational analysis of variance (PERMANOVA). Aligned rank transformed data ANOVA post hoc Tukey comparisons were used to test for significant differences among light qualities. Significant differences among light quality treatments at each biomass density level were tested by one-way ANOVAs with

post hoc Tukey's tests or Kruskal-Wallis test with post hoc Dunn comparisons when the assumption of normality was not met. Differences were considered statistically significant when $P < 0.05$.

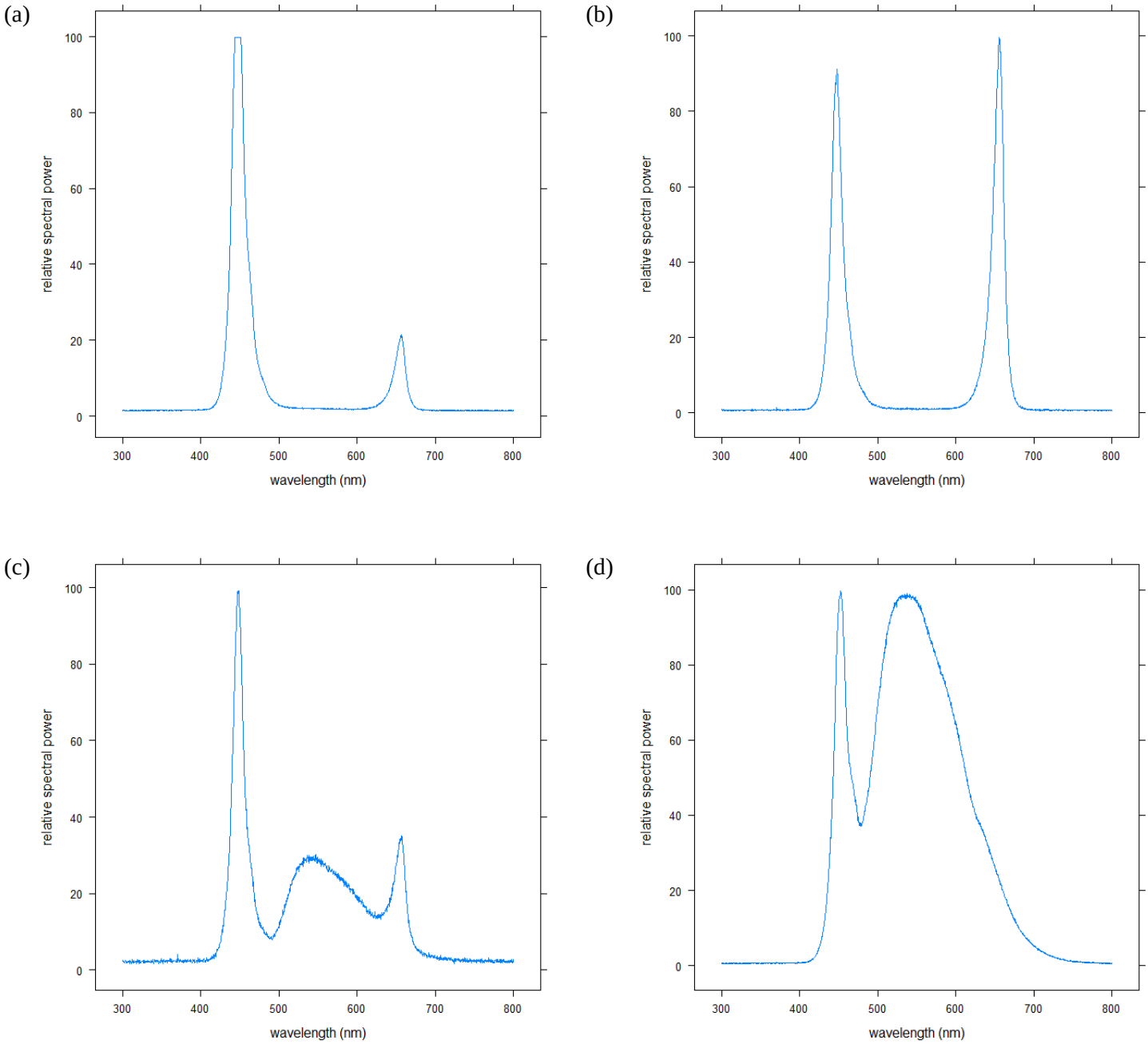

Figure 1. Relative spectral distributions of the four light qualities used in this study: (a) high blue : red, (b) medium blue : red, (c) high blue : green : red and, (d) white. Blue light refers to the waveband from 400-485, green light from 485-630 nm and red light from 630-700 nm.

2.4. Light intensity

This experiment used a 4 x 2 factorial design (light intensity x biomass density) to test the effects of light intensity on growth in high- and low-density *A. taxiformis* tetrasporophyte culture. The two biomass density levels used were 0.5 and 2.0 g FW L^{-1}. The light intensities used were 30, 60, 90, and 120 μmol photon m^{-2} s^{-1}. This broad range of intensities was selected to determine an inflexion point above which growth rates decline and to see if high density cultures benefit from high intensity. 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. In all lights, the spectral composition was maintained at the W light quality (see Figure 1), which was selected due to the high growth rates obtained with this treatment in the previous light quality experiment and its status as the most common light spectra used for indoor cultivation. For the experiment, 0.25 or 1.0 g FW of *A. taxiformis* were placed in Erlenmeyer flasks with 500 mL of f/2 enriched UV-sterilized natural seawater (salinity 33ppt, pH 8.2) and incubated in the FitoClima with controlled temperature and photoperiod (22º C and 12:12 h respectively). Culture flasks were supplied with constant bottom aeration to provide water motion and supply carbon for growth. 5 replicates were used per treatment. Algae were incubated for 3 weeks with the first week considered as the acclimatory period. At the end of each week algae were centrifuged (30 seconds, 3000 RPM), weighed, and returned to refreshed media at the experimental stocking densities. The DGR was calculated using the same formula described above. Data analysis was conducted in the program R. Permutational analysis of variance (PERMANOVA) was used to test for significant differences in DGR at different factor levels and factor interaction. Aligned rank transformed data ANOVA post hoc Tukey comparisons were used to test for significant differences between irradiance levels. Significant differences among light quality treatments at each biomass density level were tested using one-way ANOVA with post hoc Tukey's tests. Differences were considered statistically significant when $P < 0.05$.

3. RESULTS

3.1. Photosynthesis-irradiance curves

The PI curves at each biomass density are presented in Figure 2, with the photosynthetic parameters obtained from fitting the Platt et al. (1980) model to each curve in

Table 2. Saturating irradiances ranged from 13.98 µmol photon m^{-2} s^{-1} at 0.1 g FW L^{-1} to 49.95 µmol photon m^{-2} s^{-1} at 1.0 g FW L^{-1}; however, differences were determined not to be statistically significant. Maximum photosynthetic rates were similar among all biomass densities. Significant differences in the slope during the light limited phase of the curves were observed, with a significantly higher mean at 0.1 g FW L^{-1} than at 0.5, 1.0, and 2.0 g FW L^{-1}. The adjustment of the model was strong at all biomass densities (r^2>0.80). These curves were used as the basis for selecting 30 µmol photon m^{-2} s^{-1} as the controlled irradiance level in the subsequent experiment testing the effects of various light qualities on growth.

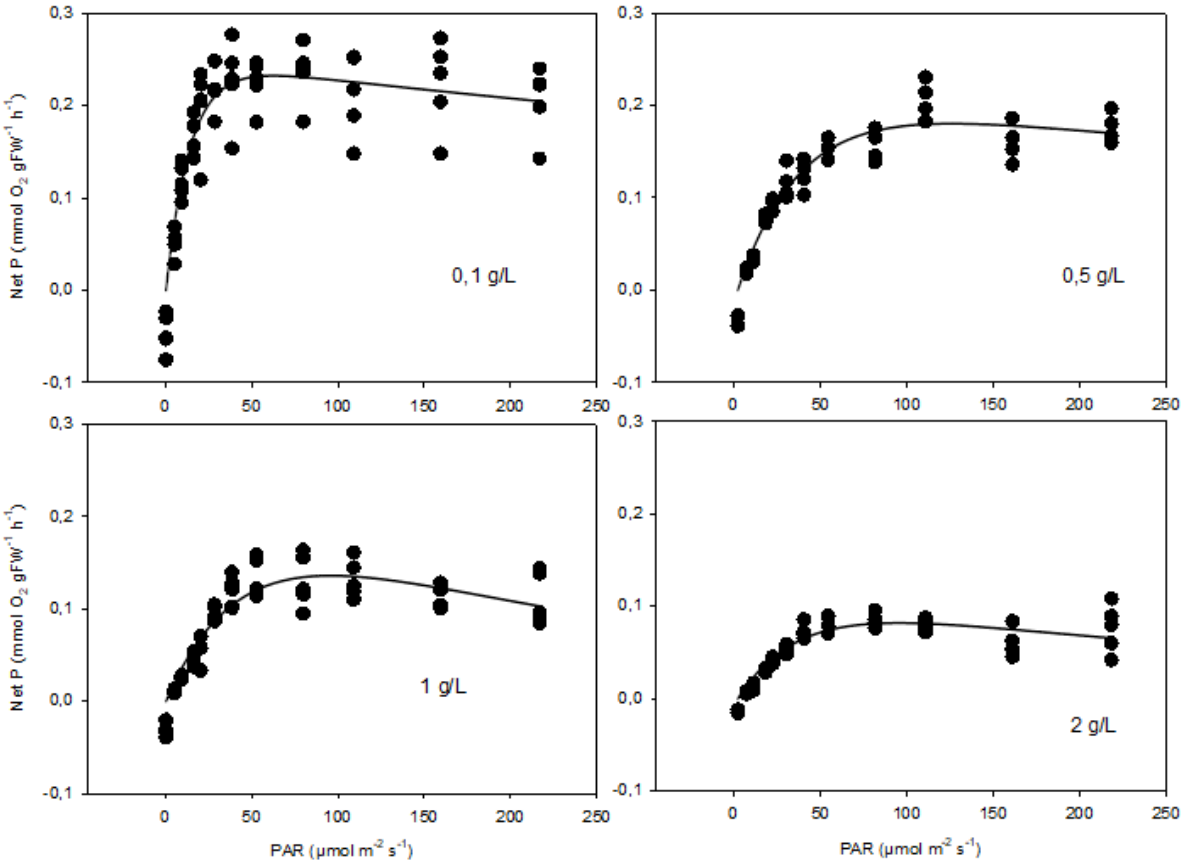

Figure 2. Photosynthesis-irradiance curves of *A. taxiformis* at biomass densities 0.1, 0.5, 1.0 and 2.0 g FW L^{-1} (n=5, except for the 0.5 g FW L^{-1} in which n=4). Curves were fitted with the Platt et al. (1980) model.

Table 2. Photosynthetic parameters obtained from the adjustment of the model equation of Platt et al. (1980) to the observed PI data for *A. taxiformis* at different biomass densities. Values are means ± se (n=5, except for the 0.5 g FW L^{-1} in which n=4, $p<0.001$). P_{max} is the maximum photosynthetic rate (mmol O_2 gFW^{-1} h^{-1}), α is the ascending slope at limiting irradiance (µmol O_2 g FW^{-1} h^{-1}(µmol photon m^{-2} s^{-1}) $^{-1}$), Ik is the saturation irradiance (µmol photon m^{-2} s^{-1}) and r^2 is the coefficient of determination of the model adjustment to the data. Different letters indicate significant differences between treatments ($p<0.05$).

Biomass density (g L^{-1})	**P_{max}**	**α**	**Ik**	**r^2**
0.1	0.25^{a} ±0.02	0.0178^{a} ±0.0019	13.98^{a} ±1.79	0.8499
0.5	0.21^{a} ±0.03	0.0057^{b} ±0.0005	37.12^{a} ±6.55	0.9251
1.0	0.22^{a} ±0.07	0.0045^{b} ±0.0004	49.95^{a} ±16.26	0.8683
2.0	0.11^{a} ±0.02	0.0030^{b}±0.0003	37.90^{a} ±8.55	0.8653

3.2. Light quality

The growth rates of *A. taxiformis* cultivated under different light spectral distribution at two biomass density conditions are plotted in Figure 3. Overall, growth rates varied from 1.50 to 9.29 % day^{-1} during the 14 days following the acclimation period. The mean growth rate for replicates stocked at 0.5 g FW L^{-1} was 7.76±0.67, 7.69±1.16, 5.34±0.56, and 4.95±1.02 % day^{-1} under HighBGR, W, BR, and HighBR light respectively. For replicates at 2.0 g FW L^{-1} mean growth rate was 3.10±0.34, 2.95±0.22, 1.96±0.19, and 1.87±0.23 % day^{-1} under W, HighBGR, BR, and HighBR light respectively. Growth rates differed significantly between light qualities and biomass densities with a significant interaction between light quality and biomass density (PERMANOVA; $P < 0.001$). Aligned rank transformed ANOVA post hoc Tukey comparisons of light qualities showed that under W and HighBGR light growth differed significantly from BR and HighBR light ($P < 0.0001$). There were no significant differences observed in DGR between W and HighBGR, nor between BR and HighBR light qualities. The significant positive effect on growth of W and HighBGR compared to BR and HighBR treatments was observed among replicates at both 0.5 (ANOVA post hoc Tukey test for quality, $p < 0.05$) and 2.0 g FW L^{-1} (Kruskal-Wallis post hoc Dunn test for quality, $p < 0.05$).

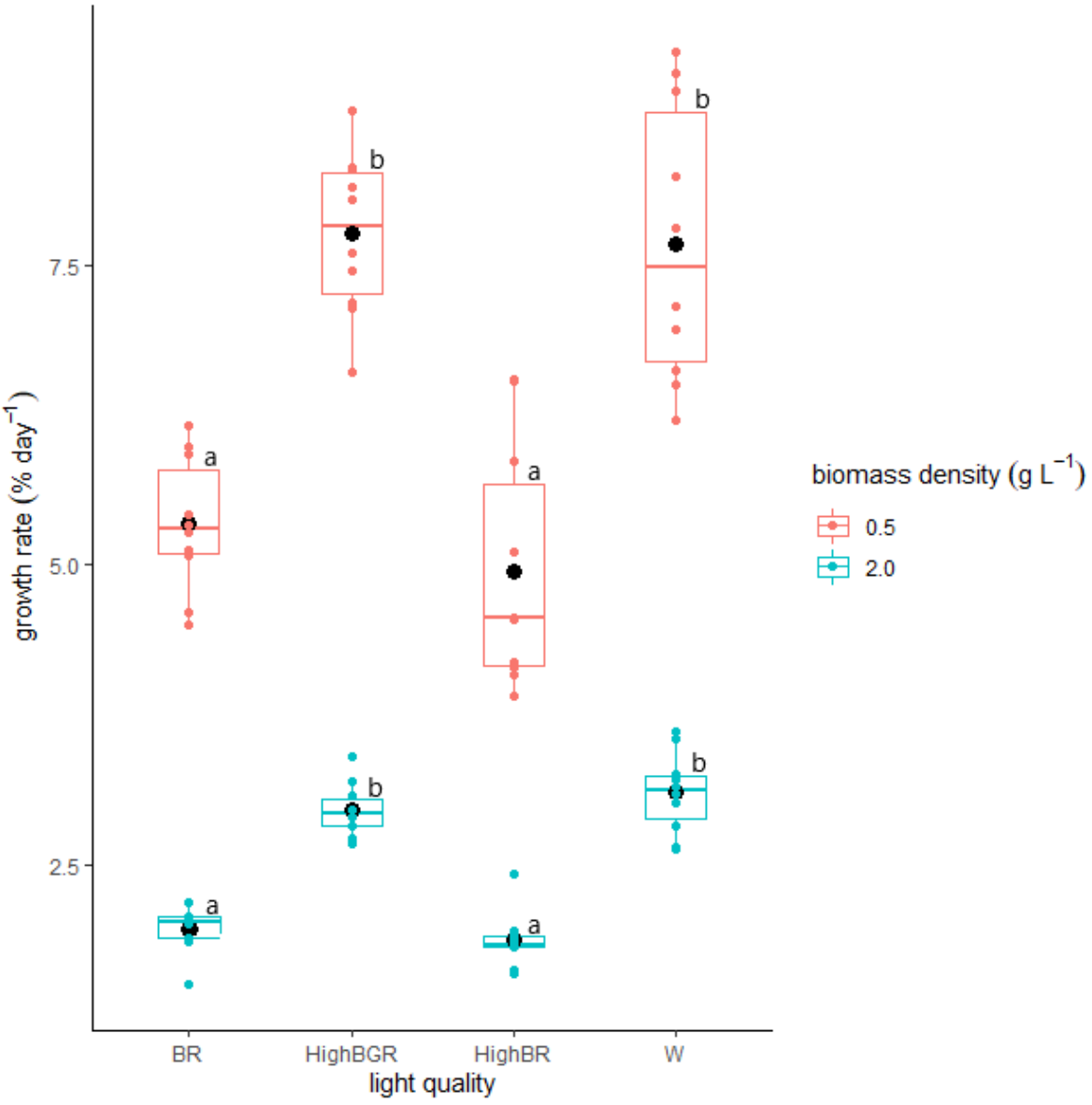

Figure 3. Growth rates of *A. taxiformis* subjected to different light quality and biomass density conditions. Boxes represent first, median, and third quantiles. Black points represent means. Points which lie beyond the whiskers are outliers. Letters indicate significant differences within each biomass density level (P<0.05).

3.3. Light intensity

The growth rates of *A. taxiformis* cultivated under different light intensity and biomass density conditions are plotted in Figure 4. Overall, growth rates varied from 3.91 to 23.16 % day^{-1} during the 14 days following acclimation. The mean growth rate for replicates stocked at 0.5 g FW L^{-1} was 10.91±1.03, 14.63±1.69, 16.09±3.03, and 16.96±1.59 % day^{-1} under 30, 60, 90 and 120 µmol photon m^{-2} s^{-1} respectively. For replicates at 2.0 g FW L^{-1}

mean growth rate was 5.25±1.59, 6.62±1.17, 6.66±1.17, and 6.19±0.91 % day^{-1} under 30, 60, 90 and 120 μmol photon m^{-2} s^{-1} respectively. DGR differed significantly between light intensities and biomass densities with a significant interaction between intensity and biomass density (PERMANOVA, P-values < 0.001). Most of the variation in DGR was explained by density (r^2=0.80), followed by irradiance (r^2=0.06), and the interaction effect (r^2=0.03). Aligned rank transformed ANOVA post hoc Tukey comparisons showed that DGR differed significantly under 30 μmol photon m^{-2} s^{-1} compared to 60, 90, and 120 μmol photon m^{-2} s^{-1} (P-values < 0.0001). No significant differences were observed among 60, 90, and 120 μmol photon m^{-2} s^{-1} conditions. Subsequently one-way ANOVA with post hoc Tukey comparisons was performed for each density level in sequence. At biomass density 0.5 g FW L^{-1} DGR differed significantly in the 30 μmol photon m^{-2} s^{-1} treatment compared to 60, 90, and 120 μmol photon m^{-2} s^{-1} treatments (P-values < 0.001) with no significant differences among 60, 90, and 120 μmol photon m^{-2} s^{-1} treatments; however, at 2.0 g FW L^{-1} average DGR declined in response to irradiance above 90 μmol photon m^{-2} s^{-1} and no significant differences were observed between 30 and 120 μmol photon m^{-2} s^{-1}.

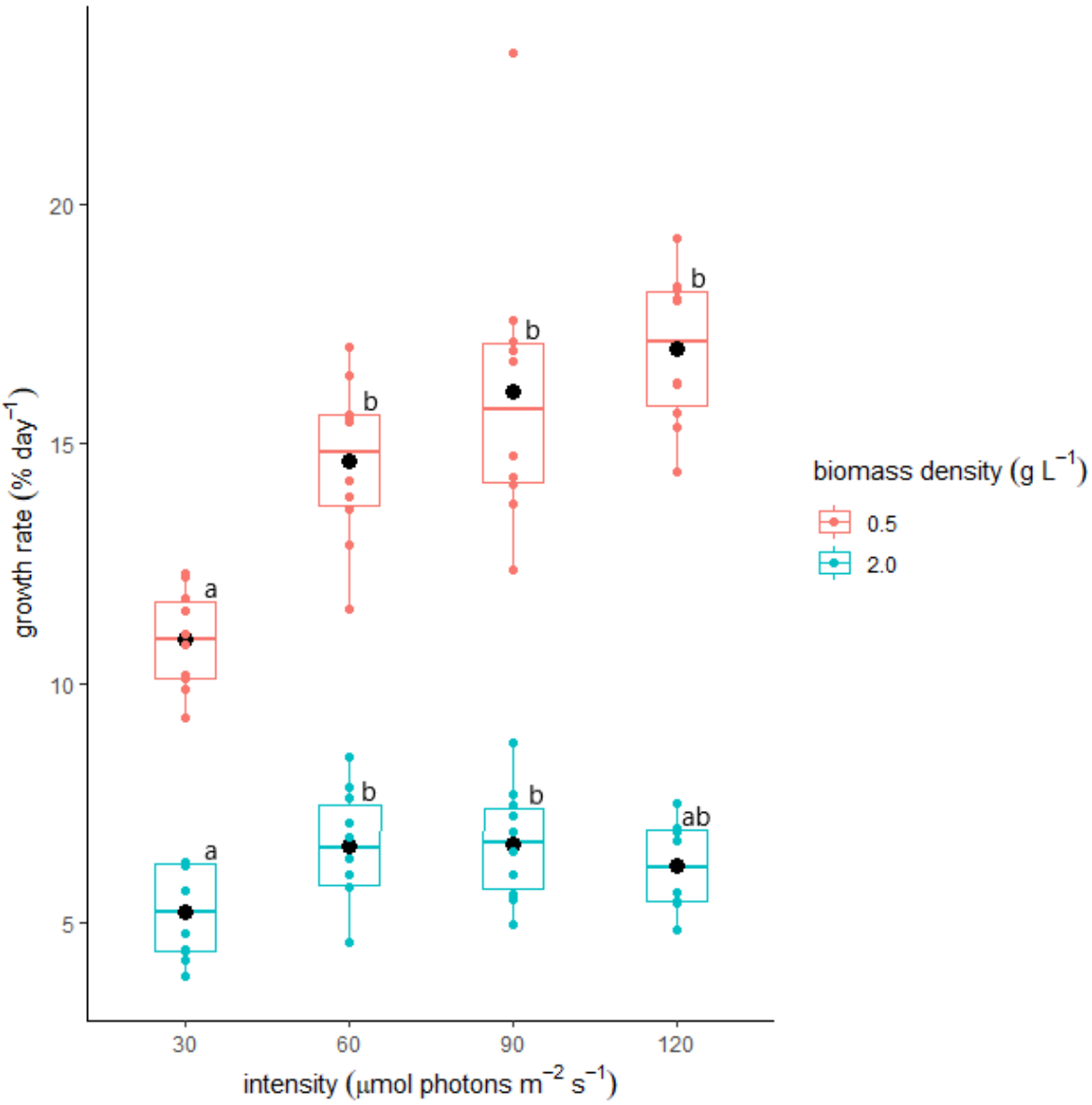

Figure 4. Growth rates of *A. taxiformis* subjected to different light intensity and biomass density conditions. Boxes show first, median, and third quantiles. Black points represent means. Points which lie beyond the whiskers are outliers. Letters indicate significant differences within the same biomass density level (P<0.05).

4. DISCUSSION

The results presented here offer compelling evidence that light spectra which include intermediate wavelengths promote high growth in *A taxiformis* compared to spectra which do not include these wavelengths. The higher performing W and HighBGR light qualities each comprise continuous wavebands which include intermediate wavelengths (485-630nm) whereas BR and HighBR qualities both consist of discrete wavebands of blue and red light.

Contrary to expectations we did not find evidence to indicate that growth was significantly different under the spectra with differing ratios of blue to red light. These results contrast with Monro and Poore (2005) who found that light quality treatments with proportionately more blue light produced compact phenotypes with more growing meristems and faster growth in *A. armata*, while a low ratio of blue to red light was correlated with lower growth. Although the present study did not evaluate morphological characteristics, any potential phenotypic variation between the spectra with different ratios of blue and red light did not translate into increased algae growth rates. Two factors likely contribute to explaining the disagreement between Monro and Poore and the present study: firstly, genotypic variation between *A. armata* and *A. taxiformis* and secondly, the difference in the dynamic nature of the light environment in tumble culture caused by algae circulation and self-shading compared to the stable light conditions found in the static culture used in Monro and Poore. Seaweed in tumble culture effectively acts as non-neutral density filters, preventing those wavelengths which are reflected or absorbed by the seaweed from penetrating deeper into the culture unit. Thus, the quantity and quality of light that seaweed is exposed to as it moves is markedly different from surface conditions and furthermore may become highly irregular as individual thali can experience chaotic motion within the culture medium.

The results point to the likelihood that the positive effect on DGR of light qualities containing intermediate "green" wavelengths is a result of the high content of phycobilin pigments characteristic of rhodophytes. Our results align with the findings of Leukart & Luning (1994) demonstrating higher growth under green compared to red and blue light in several red algae species and with those of Godínez-Ortega et al. (2008) who found that *Halymenia floresii* exhibited higher growth rates in green light than in red and blue light. These findings agree well with previous studies showing that green light is harvested efficiently by phycobilisomes (Lobban and Harrison, 2014; Borlongan et al., 2020). The growth rates we observed in the light quality experiment are consistent with values reported previously in the literature (Mata et al., 2017). The interaction between light quality and biomass density was observed to be very weak, with both biomass densities tested exhibiting the same effects of light quality on growth. Hence, within this range of biomass densities it appears the growth stimulating effect of intermediate wavelengths on *A. taxiformis* are not majorly impacted by the increasingly dynamic light environment resulting from algae self-shading and light attenuation associated with increased biomass density. Our findings that growth rates were higher under qualities with green light can be understood in relation to the

natural environment of *A. taxiformis* for which the pigment profile of the seaweed has been adapted. Intermediate wavelengths are relatively powerful in natural sunlight. Green light penetrates deeper in the water column than red light which provides phycobilin pigment rich Rhodophytes capable of capturing this light efficiently with an adaptive advantage.

Fulfilling spectral requirements to achieve the most culture suited phenotype is necessary to optimize *Asparagopsis* culture. This study indicates that *A. taxiformis* cultivation efforts should use light qualities which include wavelengths between 485-630 nm. Further research should investigate if growth rates can be increased by boosting the relative spectral power of green light. While no differences in growth rates under treatments with differing ratios of blue and red light were observed in this experiment, more research is needed to examine the effects of spectra with a broader range of ratios of blue and red light than those utilized here. Further testing of different modulations of relative power of spectral components could reveal effects on *A. taxiformis* growth. Additional studies are also needed to assess the effects of light quality on pigment composition which could provide valuable information about the capacity of the seaweed in adapting to different light qualities. Identifying a light spectral distribution which provides both efficient photochemistry and potential growth-stimulating physiological regulatory effects is a major opportunity to advance the state of the art of *Asparagopsis* cultivation.

DGR responded positively to increasing light intensity, with mean growth rates significantly lower in 30 μmol photon m^{-2} s^{-1} than in all other irradiances. Notably, *A. taxiformis* growth rates did not decline at any of the high light intensities tested, which indicates a capacity of the species to photoadapt. In view of the PI curves, which indicated that photoinhibition occurs at irradiances above 30 μmol photon m^{-2} s^{-1}, the nearly linear increase of growth rates with intensity affirms previous research indicating dynamic photoinhibition in *Asparagopsis* (Mata et al., 2006). In dynamic photoinhibition algae utilize adaptive photoprotective mechanisms to prevent excess absorption of light energy from damaging the photosynthetic apparatus (Krause and Weiss, 1991; Hader and Figueroa, 1997). The primary mechanism is non-photochemical quenching which increases the proportion of light energy which is diffused as heat by the light harvesting complex relative to the portion used in photochemistry (Müller et al., 2001). The current study highlights the ability of *A. taxiformis* to adapt to high light conditions, indicating that irradiances above 30 μmol photon m^{-2} s^{-1} and as high as 120 μmol photon m^{-2} s^{-1} may be suitable for cultivation. The present study contrasts with previous findings by Oza (1989) that *A. armata* showed maximum

growth at 1200 lux (~25 μmol photon m^{-2} s^{-1}). An important issue to resolve for further study is if the high growth rates described here can be sustained over longer periods of time at these intensities.

Interestingly, a plateau in growth rate was observed in *A. taxiformis* at 2.0 g FW L^{-1} above 90 μmol photon m^{-2} s^{-1}while growth continued to increase with intensity at 0.5 g FW L^{-1}. This was contrary to expectations that negative impacts of high irradiance on growth would be observed first in low density culture, due to the self-shading associated with biomass density that would mitigate irradiance induced stress and the activation of protective non-photochemical quenching mechanisms to reduce light harvest efficiency. This data is likely attributable to the unexpected development during cultivation of opacity in the culture media and its effect on the actual light conditions experienced by the algae.

Every replicate in the intensity experiment at irradiance levels of 60, 90, and 120 μmol photon m^{-2} s^{-1} became visibly depigmented after 5 days of cultivation, while at 30 μmol photon m^{-2} s^{-1} biomass retained a bright red coloration. A marked increase of the opacity of the culture media was observed concurrent with the decoloration of algae, with reductions in transparency most acute at 2.0 g FW L^{-1}. The opacity was likely the result of the shedding of organic compounds by light stressed algae which obstruct light transmission within the media. This phenomenon had a more substantial effect in higher biomass density cultures where the shedding of organic compounds in a closed system rapidly increased particulate concentration and reduced transmissibility of light in the media. Macroalgae bleaching is a phenomenon typified by pigment degradation and loss (Scrosati and DeWreede, 1998). Irradiance has been linked to bleaching in macroalgae, including in rhodophytes (Martone et al., 2010; Quintano et al., 2017). Some authors have proposed that bleaching functions as an acclimatory mechanism for enabling continued growth in high light conditions (Irving et al., 2004). However, there are usually negative consequences associated with bleaching including chronic tissue damage, growth rate reduction, and biomass loss (Scrosati and DeWreede, 1998; Martone et al., 2010). This experiment indicates *A. taxiformis* partial depigmentation occurs in irradiances of 60 μmol photon m^{-2} s^{-1} and higher but did not negatively affect the growth rates at least during the two following weeks. It seems likely that the optic density of the culture media acted as a confounding variable; therefore, data for the intensity experiment should be interpreted with extreme caution. These confounding effects may be prevented using a more gradual, multi-step acclimation to high light intensities.

5. CONCLUSION

Understanding the response of seaweed growth rate to light quality and intensity at different biomass densities is essential to managing cultivation and determining culture protocols. Optimizing growth rates is key to the productivity and sustainability of aquacultural systems. We demonstrated that *A. taxiformis* tetrasporophyte growth rates respond positively to light qualities containing wavelengths in the 450-630 nm range and were not affected by the different ratios of blue and red light evaluated. High growth rates were achieved under high light intensity, regardless of the biomass density, presenting the possibility of achieving higher yields with cultivation under 90 μmol photon m^{-2} s^{-1}. The findings presented here could be built upon by examining the effects of light qualities with a higher proportion of green light and the effects of light qualities with a wider range of ratios of blue and red light. On the light intensity side, the use of a gradual multi-step acclimation regimen to higher light intensities to facilitate an adaptive response and reduce light stress. Finally, pigment composition plasticity should have been evaluated in *A. taxiformis* tissue to understand how the pigment composition changed in response to light quality and intensity.

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