Reproduction, growth and chemical composition of *Ulva* sp. in response to different light treatments

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Abstract

In this study, *Ulva* sp. collected on Helgoland were subjected to one of four light treatments consisting of different light colour and daylength: 24h blue light, 24h white light, 16h blue light and 16h white light. All algae were kept in a temperature controlled dark room on Helgoland at 15 °C. Over the course of 44 days growth rate, photosynthesis (via oxygen spot optodes) and reproductive area were measured. At the end of the experiment the antioxidant activity of extracts from the algae was measured using the ABTS radical decolourization assay. In addition chlorophyll and carotenoid concentration were analysed for all treatments. The aim was to identify conditions with minimal reproduction and maximal growth and antioxidant activity. These parameters were of interested for potential applications of *Ulva* in the development of alternative packaging material. White light treatments had higher growth rates than blue light (12.6 and 10.6 % d^{-1} vs. 4.17 and 2.69 % d^{-1}). However this is likely due to differences in light intensity rather than colour. Similarly, reproduction only occurred in white light treatments and irrespective of daylength. Since the algae grown in white light also were biggest, this trend is also not necessarily attributable to light colour and more likely a consequence of size. Antioxidant activity was highest in algae grown under 24h-BL. While this is also at least partially a consequence of light intensity, light colour also seems to play a role. Pigment content followed a similar trend with treatments under low light intensity (blue light) containing more pigments than those under high intensity (white) light. The 24h white light treatment was an exception to this and did not differ significantly from treatments under blue light. The implication of light colour and intensity, both daily and short term, for algal physiology in general and algae in this study in particular are reviewed. Potential strategies to manipulate antioxidants in the context of commercial applications such as biodegradable, sustainable packaging are discussed.

Zusammenfassung

Für diese Studie wurden auf Helgoland gesammelte Algen der Gattung *Ulva* vier Kombinationen aus verschiedenen Tageslängen und Lichtfarben ausgesetzt: 24h blaues Licht, 24h weißes Licht, 16h blaues Licht und 16h weißes Licht. Alle Experimente wurden in einem Klimaraum bei 15 °C der Biologischen Anstalt Helgoland (BAH) durchgeführt. Über einen Zeitraum von 44 Tagen wurden Wachstumsrate, Photosynthese (über Sauerstoffoptoden) und reproduktive Thallusfläche bestimmt. Nach dem Ende des Experiments wurde die antioxidative Aktivität der Algen mit Hilfe der ABTS-Radikalentfärbungsmethode gemessen. Zusätzlich wurden Chlorophyll- und Carotinoid-konzentrationen aufgezeichnet. Ziel war es, Anbaubedingungen mit minimaler Fortpflanzung, maximalem Wachstum und maximaler antioxidativer Aktivität zu identifizieren. Diese Faktoren sind für die Verwendung von *Ulva* bei der Entwicklung alternativer Verpackungsmaterialien besonders interessant. Unter weißem Licht kultivierte Algen besaßen höhere Wachstumsraten als solche unter blauem Licht (12.6 und 10.6 % d^{-1} bzw. 4.17 und 2.69 % d^{-1}). Diese Unterschiede lassen sich vermutlich auf unterschiedliche Lichtintensitäten und nicht die Lichtfarbe zurückführen. Reproduktives Gewebe wurde nur unter weißem Licht und unabhängig von der Tageslänge beobachtet. Da diese Algen auch die größten Thalli waren, ist diese Beobachtung nicht zwangsläufig auf die Lichtfarbe, sondern eher auf die Thallusgröße zurückzuführen. Die antioxidative Aktivität war am größten unter 24h blauem Licht. Dafür ist vermutlich die Lichtfarbe verantwortlich, aber die Lichtintensität spielt ebenfalls eine Rolle. Die Pigmentierung der Algen folgte einer ähnlichen Tendenz: Algen unter geringer Lichtintensität (blaues Licht), enthielten mehr Pigmente als solche, die unter hoher Lichtintensität (weißes Licht) kultiviert wurden. Algen die 24h weißem Licht ausgesetzt waren, bildeten eine Ausnahme. Ihr Pigmentgehalt unterschied sich nicht signifikant von dem der Algen unter blauem Licht. Die Auswirkungen von Lichtfarbe und Intensität auf die Physiologie von Algen im Allgemeinen und den in dieser Studie untersuchten Algen im Besonderen werden besprochen. Möglichkeiten zur Manipulation von antioxidativen Stoffen in Algen für die kommerzielle Nutzung, zum Beispiel für biologisch abbaubare, nachhaltige Verpackungen, werden diskutiert.

1. Introduction

Humans have long been utilising algae. Remains found in caves have shown that even 14 000 years ago algae were part of the diet of ancient humans (Dillehay et al., 2008). Seaweed aquaculture is believed to have started much later. The first sources mentioning seaweed aquaculture come from 16th century Korea (Haeng, 1531) and Japan where farmers submerged bamboo racks for kelp spores to settle on (Borgese, 1980). Harvesting seaweeds also used to play a role in the coastal areas of Europe. Coastal residents of Ireland and Scotland in particular have used seaweed as animal feed or for human consumption, i.e. as dulse, tangle or laver bread (Naylor, 1976). Over the course of the 20th century however, seaweeds have largely vanished from western diets (Ferdouse et al., 2018). On the other hand, the popularity of Asian cuisine all over the world today has led to a reintroduction of seaweed into the everyday lives of many people previously less accustomed to this form of seafood in their diet. Additional popularity is gained from the image of seaweed as so-called "superfood" (Ferdouse et al., 2018). However, seaweed, or more specifically products derived from seaweeds, is much more prevalent even in western countries than many people realise. Gelling agents, such as carrageenan (E-407) and agar (E-406), are extracted from seaweeds and then used as food additives or in toothpaste and cosmetics (Renn, 1997).

The discovery of a multitude of bioactive compounds within seaweeds has further increased the commercial interest in seaweed in general as well as in seaweed aquaculture in particular. Many of these compounds have potential medical implications, such as antiviral, antibacterial or anti-inflammatory properties (Smit, 2004; Holdt and Kraan, 2011). Studies have shown that the possibility for seaweed extracts are varied ranging from inhibition of the growth of cancer cells to possibly preventing HIV transmission (Witvrouw and De Clercq, 1997; Yamada *et al.*, 2000; Smit, 2004).

For agar and carrageenan production as well as other commercial purposes, mostly tropical seaweeds, such as *Kappaphyus alvarezi* and *Euchema spp*. are chosen (Ferdouse et al., 2018). These species usually have very high concentrations of the desired compounds. Moreover, they have the benefit of year round growth due to the lack of seasons in the tropics. This makes cultures of these species, in the tropics, easy to maintain, since no additional lighting or heating is required. In temperate regions predominantly kelps such as *Undaria pinnatifida* or *Saccharina latissima* are cultured (Ferdouse et al., 2018). In comparison to Asia, European seaweed aquaculture still lies in its infancy. In a global context however, seaweed does play a significant economic role. In 2015 the global seaweed industry harvested more than 30 million tons of biomass with an estimated value of USD 6 Billion (Ferdouse et al., 2018).

In addition to the aforementioned examples for cultivated species of red and brown seaweeds, green seaweeds (Chlorophyta) have also shown potential. Among these are species of the genus *Ulva. Ulva* has been known for its incredibly high growth rates. This can lead to the occurrence of green tides, huge *Ulva* blooms covering entire beaches and huge areas of ocean (Keesing et al., 2011; Smetacek and Zingone, 2013); a phenomenon predicted to increase with ongoing climate change (Smetacek and Zingone, 2013; Gao et al., 2017a). While a nuisance to some, the phenomenal growth performance of *Ulva* makes it an interesting candidate for commercial cultivation. But the species in this genus also have other benefits aside from their fast growth: Like many other algae *Ulva* spp. contain a host of bioactive compounds with potential applications in medicine and industry (Wang et al., 2014). Among these are the ulvans, a group of highly branched polymers of soluble dietary fiber and sulphated polysaccharides with anti-inflammatory properties (Holdt and Kraan, 2011) and

potential in the treatment of gastric ulcers (Lahaye and Robic, 2007). Additionally, the components of ulvan (i.e. iduronic acid) can be used as the starting point for the synthesis of fine chemicals (Lahaye, 1998) and rhamnose is used in the synthesis of valuable aroma compounds (Holdt and Kraan, 2011). In terms of potentially interesting proteins, *Ulva* sp. contains Lectin which exhibits antibiotic properties (Holdt and Kraan, 2011). The species also contain a variety of antioxidants which serve as an added benefit from a human health perspective (Holdt and Kraan, 2011; Hassan et al., 2011) and are known to be relevant in preventing or mitigating metabolic syndrome (Mohamed, 2014). Especially the ulvans are known for their potent antioxidant activity (Qi et al., 2010; Qi and Sun, 2015). Antioxidants extracted from marine macroalgae are especially interesting, since some show higher activity than expensive synthetic alternatives (Thomas and Kim, 2013).

These properties make algae in general, and *Ulva* in particular, interesting in the search for alternative biodegradable packaging material. Especially in the context of food packaging, the antimicrobial compounds and high antioxidant activity in algal extracts have the potential to enhance the function of the packaging by increasing the shelf life of the food. Depending on the packaging formula, the packaging itself can be edible and serve as a nutritious and healthy bonus to the food. Several pilot studies have been conducted in this area. Shin et al. (2012) produced edible films on a grapefruit seed basis with added extracts from red algae to coat cheese and bacon. The algal extracts imbued these films with significant antimicrobial properties (Shin *et al.*, 2012; Song *et al.*, 2013). In general, extensive research has been done on edible films incorporating alginates, carrageenan or agar (Cha and Chinnan, 2004). Many of the developed films had antimicrobial effects or other beneficial effects on the coated food products. The Indonesian company Evoware (Evoware 2019) has already brought the concept of algae based packaging to marketability. So far the start-up produces packaging for food but also for sanitary products. Their spice satchels, coffee packs or soap packaging are especially interesting: The wrapping is edible and dissolves upon contact with hot water making unwrapping unnecessary and leaving behind zero waste. Any beneficial biochemical compounds, like antioxidants, are added to the food with the packaging.

Aside from the biochemical potential of *Ulva*, these species can and have been used successfully in a number of different functions. Due to the high potential for growth, especially in eutrophic conditions, *Ulva* has been utilised for bioremediation. Sode et al. (2013) discussed and successfully tested the possibility of using *Ulva lactuca* to treat sewage. More commonly *Ulva* has been tested for use in Integrated-Multitrophic Aquaculture (IMTA) systems (Lawton et al., 2013). Here it is deployed as nutrient extractors to clean the effluent of aquaculture operations, such as fish farms. This has been proven especially successful in South Africa where *Ulva* and kelp is used in abalone farms both to clean the water as well as to serve as food source for the cultured abalone (Naidoo et al., 2006; Bolton et al., 2009). Other studies have shown the benefits of *Ulva lactuca* in shrimp culture (Brito et al., 2014) and tilapia feed (Ergün et al., 2008). Several studies have furthermore demonstrated that *Ulva* has a high affinity for heavy metals, a property that is even retained by dead material (Suzuki, et al., 2005; Ibrahim et al., 2016). This opens up the possibility to use these algae to clean contaminated coastal areas.

However, *Ulva* cultivation faces a major problem and that is the periodic nature of reproduction of members of this genus (Smith, 1947; Lüning et al., 2008). Because reproduction leads to the disintegration of parts or even the entire thallus, this can seriously limit biomass production. The time between reproduction events is mainly dependent on two external factors: Light and temperature. Lüning et al. (2008) reported that continuous light prevented gamete release in *Ulva*

pseudocurvata. Temperature shock, on the other hand can be used to trigger reproduction (Gao et al., 2017b). Kalita and Titlyanov (2011) demonstrated that an increase in temperature greatly reduced the time between reproductive events (30 to 5 days) in *Ulva fenestra*. They furthermore showed that under unfavourable conditions the length of the reproductive cycle increased.

Reproduction is also controlled by at least one internal factor: Substances produced at the base of the thallus have been shown to inhibit reproduction (Bold and Wynne, 1985; Stratmann et al., 1996). Their concentration decreases with distance from the base of the thallus. Reproduction thus occurs once the concentration is low enough, i.e. the thallus has grown large enough or produced less of the inhibition compounds. These substances are also secreted into the water column and can slow or prevent the reproduction of co-cultured blade material (Gao, 2016).

Light in general is known to have pronounced effects on the physiology of higher plants but also on algae (Kami et al., 2010). These processes are collectively known as photomorphogenesis and control a variety of physiological processes. Algae are similar to the higher plants equipped with a number of photoreceptors that allow them to sense various light parameters, such as colour (Hegemann, 2008). Light colour seems to be an especially important parameter when regulating reproduction. In Laminarians the proportion of fertile gametophytes produced is increased under blue light and lower under red light (Lüning and Dring, 1972). It has also been demonstrated that blue light prevented gamete release in *Ulva lactuca* (Wichard and Oertel, 2010) and *Laminaria* (Lüning, 1981). However, the information on the molecular mechanisms of photoreception in marine macroalgae is still limited (Liu et al., 2017).

Beyond regulating reproduction, blue light also influences other processes in algae. Le et al. (2018) were able to show that *Ulva pertusa* grown under blue LED-light showed higher antioxidant activity than those grown under the full spectrum. The same has been demonstrated for *Rehmannia glutinosa* (Manivannan et al., 2015). It has also been theorized that blue light in particular regulates a variety of other important biochemical processes in algae, such as chlorophyll synthesis (Senger and Bauer, 1987), enzyme synthesis (Roscher and Zetsche, 1986) and gamete and spore release (Lüning, 1981; Lüning et al., 2008). In a study by Kamiya and Saitoh (2002) it was shown that blue light can influence the nutrient uptake ability of C*hlorella* mutants. In microalgae light intensity is known to influence the chemical composition. High content of polyunsaturated fatty acids (PUFA) has been reported under low light intensity and more saturated and monounsaturated fatty acids under high light intensity. The red macro alga *Tichocarpus crinitus* (Khotimchenko and Yakovleva, 2005) showed similar results. However, the trend seems less clear in macro than in microalgae. This is another example for the pronounced impact that light quality can exert on biochemical processes in algae. Light quality and intensity can therefore serve as powerful tools to influence the chemical composition of algae in culture.

The aim of this project was to identify the impact of different light regimes (colour and day length) on growth performance, reproduction and antioxidant capacity of *Ulva* sp. This study is part of the MakPak project (AWI 2019) with the overarching aim to use *Ulva* biomass to produce biodegradable sustainable fastfood packaging. The project is conducted in conjunction with the Hochschule Bremerhaven (material test, concept development) and the “Nordsee” company (development & design), a popular German seafood restaurant chain. Particular interest was taken in the identification of culture parameters with maximum growth and minimal reproduction. The following hypotheses were the focus of this study:

1. Reproduction will only occur once the algae have reached a certain size and not under 24h illumination.

2. The algae growing under white light (WL) will show higher growth rates than those grown under blue light (BL). In the long term those in the 16h-WL treatment will show the highest growth rates.

3. Extracts from algae grown under blue light will have higher antioxidant activity than those grown under white light. Among those the 24h-BL treatment should have the highest.

2. Methods

2.1 Experimental Set-up

Algae were collected on the rocky shore on Helgoland (54°11'18.2"N 7°52'25.1"E) in early April 2019. After transport to the lab, they were kept in 10 L glass jars at 15°C with 16h days (white light; Mitras Lightbar Daylight; GHL Advanced Technology GmbH & Co. KG) until the start of the experiment (24-48h). Prior to the start of the experiment, 400 pieces (1 cm length from holdfast) were cut from the basal parts of the collected algae to obtain fragments with high concentrations of the reproduction inhibiting substances. 20 fragments each were placed in a 1 L Schott flask with filtered artificial seawater (0.2 µm; 33 psu Tropic Marine) resulting in 20 flasks with 20 disks (Fig. 1). Groups of 5 flasks were then subjected to one of four treatments:

1. Blue light with a 24/0 light/dark cycle (24h-BL)
2. White light with a 24/0 light/dark cycle (24h-WL)
3. Blue light with a 16/8 light/dark cycle (16h-BL)
4. White light with a 16/8 light/dark cycle (16h-WL)

Based on studies by Fortes and Lüning (1980) and Gao (2016), the 16/8 light/dark cycle represents optimum conditions for daylength. All experiments were set up in a temperature controlled dark room at the AWI on Helgoland to ensure maximum control over the light intensity. Based on the aforementioned studies, 15 °C was chosen as the cultivation temperature. This seems to be the upper temperature optimum on Helgoland (Fortes and Lüning, 1980; Gao, 2016).

For illumination, one Mitras Lightbar Daylight (GHL Advanced Technology GmbH & Co. KG) per treatment was used (80 µmol photons m^{-2} s^{-1}; blue light 472 nm). Irradiances above 70 µmol photons m^{-2} s^{-1} are known to be the saturation point for growth in *Ulva* on Helgoland, hence an intensity slightly above this point was chosen (Fortes and Lüning, 1980). The blue lamps, unfortunately, were only able to achieve a maximum intensity of 40 µmol photons m^{-2} s^{-1}. In order to control the daily cycles and adjust the irradiance, the lamps were connected to a ProfiLux Mini through a Mitras-LB-Splitter and a Lightbar-Interface cable (all GHL Advanced Technology GmbH & Co. KG). All treatments were surrounded by opaque plastic to avoid cross contamination of light. These covers did not interfere with the temperature of the treatments.

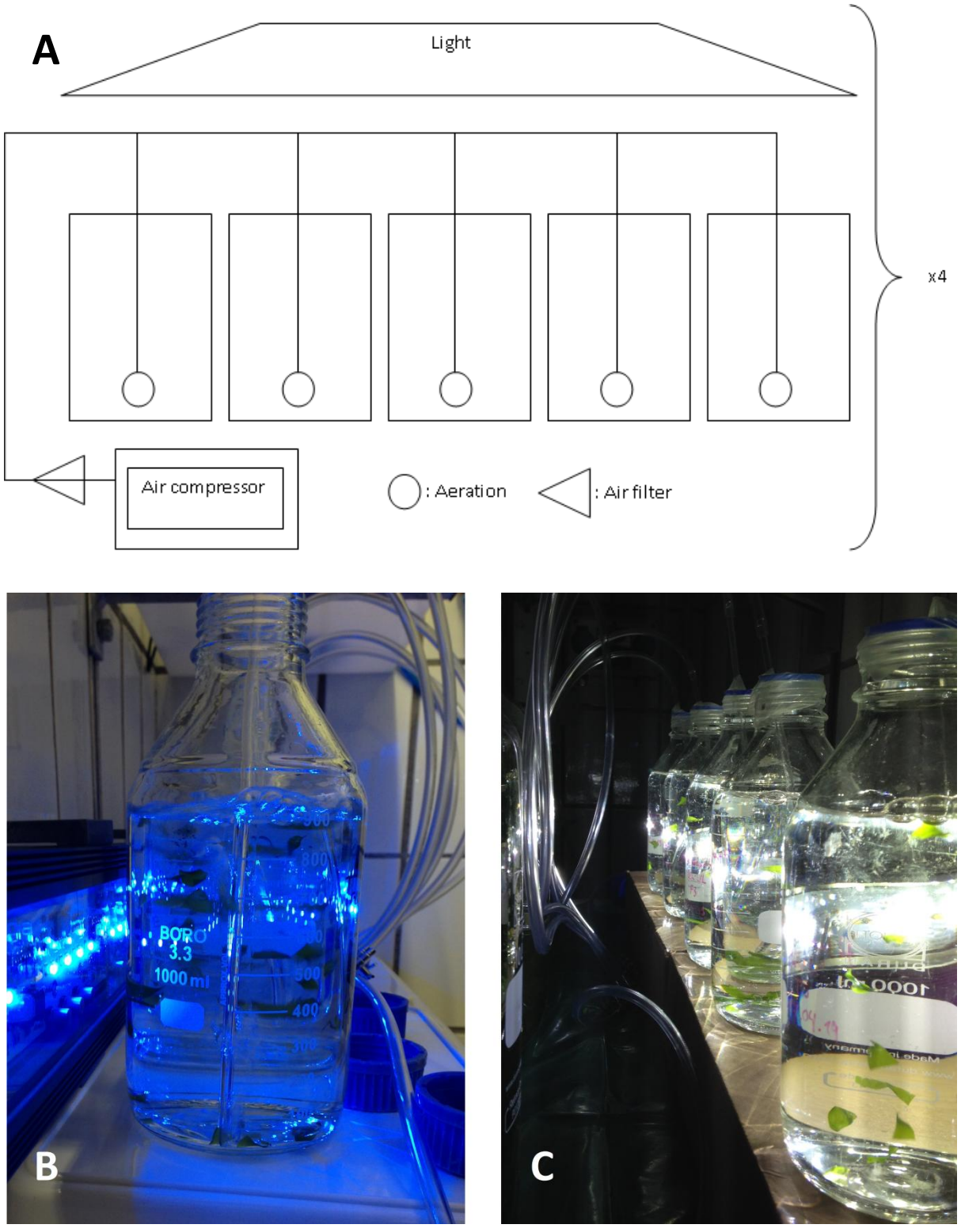

Figure 1: Schematic of the experimental set-up **(A)**. Boxes in the middle denote flasks with seawater and 20 basal disks of *Ulva* sp. **(B)** Set-up for the 24h-BL treatment. The white light visible in the background was turned off during the actual experiment. **(C)** Experimental set-up for the 24h-WL treatment.

Each treatment (5 flasks) was constantly aerated to ensure suspension of the algae, water mixing, and gas exchange (Figure 1). The air passed through an airfilter (Midisart 2000, 0.2 µm; Sartorius Stedim Biotech) to prevent culture contamination. The experiment was conducted over the course of

44 days. 40 µL of blue fertilizer (Blaukorn) were added to each flask (Lüning pers. comm.) every eight days, resulting in a concentration of ~300 µmol/L. Complete water exchanges were also performed at the same intervals.

Analytical methods

Measurements of area, weight and photosynthesis were taken every four days. For the weight measurements the algae pieces were gently dried with a paper towel before weighing on a Sartorius Secura (224-1CEU). Specific growth rates were calculated according to the following formula:

$$Growth\ rate\ \left(\%/_{d}\right) = \left(\frac{\ln(M_2 - M_1)}{t}\right) * 100 \quad (1)$$

M_1 and M_2 are the measured weight at two time points and t the time between those measurements.

In order to determine the area of each fragment all pieces were photographed on a graph paper. Using the software ImageJ v. 1.52a (Rasband, 2019) each image was converted to black and white (Fig. 2) and the area of each fragment was calculated. Absolute and relative area increases were then calculated based on this data. In this fashion, the relative proportion of reproductive area (evident from faded or missing spots on the fragments) was also recorded using the following equation:

$$Relative\ reproductive\ area\ \left(\%/_{individual}\right) = \left(\frac{rA_t}{A_t}\right) * 100 \quad (2)$$

rA_t is the reproductive area at time t and A_t the total area at time t.

In the same four day intervals, photosynthesis was measured via oxygen spot optodes (PyroScience, Firesting O2) to assess the effects of the different light treatments on the photosynthetic performance of *Ulva* sp. For these measurements, the algae material from a flask in a treatment was deposited in a 250 ml bottle filled with artificial seawater and prepared with oxygen spots as sensors. Special care was taken not to enclose any airbubbles when closing the bottles. Optodes were then fastened on the bottels at the oxygen sensors. The algae in the bottles were placed on a magnetic stirrer to ensure constant mixing during the measurement. The bottles were subsequently exposed to the light of their respective treatments. Oxygen concentration was measured using the Firesting O2 (PyroScience) connected to a laptop with the Pyro Oxygen Logger software (V3.313). Measurement time was 30 minutes or until the slope was stable. To obtain oxygen production rates, a line of best fit was fitted to the data for each measurement using Microsoft Excel. Oxygen production rates in µmol/g/s were then calculated according to the following formula:

$$Oxygen\ production\ rate\ (\mu mol/g/s) = \frac{(m * V)}{g} \quad (3)$$

m is the slope obtained from the oxygen measurement, V the volume the measurement was taken in (0.25 L in this case) and g the gram weight of material measured. Due to an error when executing the method, only results from day one and day 44 can be reported here for the oxygen production rates. On these days special care was taken to use the same ratio of material-to-bottle volume for the measurement.

At the end of the 44 days, all algae were dried for 48h at 30 °C (Memmert drying oven). This temperature was chosen to conserve most compounds interesting for packaging (i.e. antioxidants and antimicrobial substances). All samples were transferred to Bremerhaven for further analysis.

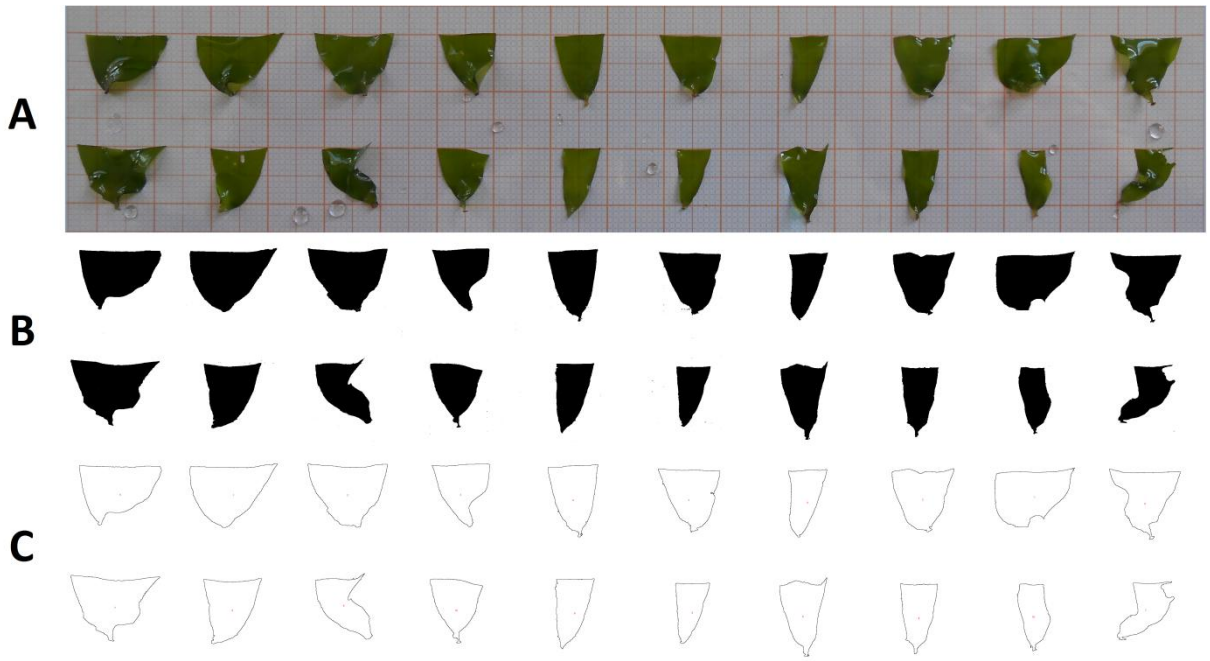

Figure 2: Fragments on 17.04.19 in the 16h-WL treatment **(A)**, after converting to black and white **(B)** and processing in imageJ **(C)**.

2.2 Preparation of Extracts

Algal extracts were prepared by grinding 0.1 g of dry algae material together with 0.3 g of sand (to increase friction) and 1 ml of Ethanol (70 %). The Ethanol was added in 0.5 ml intervals. The ground algae were then washed out of the mortar into Falcon tubes using 1 ml of Ethanol. Samples were stored on ice until extraction. The extraction took place in a water bath (GFL 1086) at 47 °C for a duration of six hours with moderate shaking. Samples were then centrifuged (Eppendorf, Centrifuge 5810 R) at 2500xg and 4 °C for 10 minutes. The supernatant was removed into a fresh Falcon tube and 2 ml of ethanol (70 %) were added to the remaining pellet. After another round of extraction in the water bath (1 hour) and centrifuging, the supernatant was added to the previously removed.

2.3 Antioxidant activity (ABTS)

The antioxidant activity was determined following the ABTS assay outlined in Re et al. (1999) with modifications for use with 96 well plates described in Torres et al. (2017; 2019). ABTS (2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid; AppliChem GmbH, CAS: 30931-67-0) was dissolved in water to create a 7 mM solution. To produce the ABTS radical solution (referred to as ABTS+), the 7 mM ABTS was mixed with potassium persulfate (Honeywell Fiuka, CAS: 7727-21-1) to create a 2.45 mM solution. This mixture was then incubated in the dark for 12-16 hours. Before measuring, this solution was diluted to an absorption of 0.70 ± 0.02 at 734 nm.

The absorbance of the empty plate was measured at 734 nm and later subtracted from the results. 20 µl of each sample were added to the wells in triplicate as well as blanks for the ABTS+ solution. Following this 280 µL of diluted ABTS+ solution were added to each well. The absorption at 734 nm was measured after an incubation time of 10 minutes on a Tecan infinite M200 plate reader.

In addition to this, a standard curve using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Aldrich Chemistry, CAS: 53188-07-1) (0-100 µg/ml) was prepared in the same fashion ($y = -0.0061x + 0.6477$; $r^2 = 0.9986$). To determine the solid content of the extract, 1.5 ml of each extract were dried at 60 °C for 12 hours (Thermo Scientfic Heraeus). Based on the Trolox standard curve, the antioxidant activity of each sample was calculated as µmol Trolox equivalent per gram dry algal extract:

$$Trolox\ Equivalent\ TE\ (\mu g/ml) = \frac{A_{734} - 0.6477}{-0.0061} \quad (4)$$

A_{734} is the measured absorbance of the sample at 734 nm. The two coefficients are obtained from the standard curve ($y = -0.0061x + 0.6477$; $r^2 = 0.9986$). Using the obtained values, they were converted into Trolox Equivalent µmol/g dry extract by the following equation:

$$Trolox\ Equivalent\ (\mu mol/g\ dry\ extract) = (\frac{TE}{250.29 g/mol} * 10^6)/g_{de} \quad (5)$$

TE is the Trolox Equivalent obtained in equation (4) converted to gram and g_{de} the measured amount of dry extract in gram.

2.4 Pigment Extraction and Analysis

Extracts for pigment extraction were prepared by grinding 0.1 g of dried algae together with 0.3 g of sand and 1 ml Ethanol (95 %) added to the mortar in 0.5 ml intervals. Ground algae were then washed into falcon tubes by adding another 1 ml of Ethanol. Extraction took place over night at 4 °C. After centrifuging (4 °C, 3000xg) for 10 minutes the supernatant was removed into a fresh falcon tube and stored at 4 °C. 1 ml of Ethanol (95 %) was added to the remaining pellet. the pellet was resuspended and again extracted over night. After centrifuging the supernatant was added to the one from the previous extraction. A 1:3 dilution was prepared from all extracts. The diluted extracts were read at 470, 649 and 664 nm on a UV-2450 Shimadzu UA6. Chlorophyll and carotenoid concentrations were calculated according to the formula developed by Lichtenthaler (1987):

$$Total\ chlorophyll\ a + b\ (\mu g/ml) = 22.24\ A_{649} + 5.24\ A_{664} \quad (6)$$

$$Chlorophyll\ a\ (\mu g/ml) = 13.36\ A_{664} - 5.19\ A_{649} \quad (7)$$

$$Chlorophyll\ b\ (\mu g/ml) = 27.43\ A_{649} - 8.12\ A_{664} \quad (8)$$

$$Total\ carotinoids\ (\mu g/ml) = 4.8\ A_{470} - 12.7\ A_{649} + 3.65\ A_{664} \quad (9)$$

A is the absorption at 470, 649 and 664 nm, respectively.

2.5 Statistical Analysis

The results for each treatment were then compared using Excel and R (v. 3.6.1, R Core Team, 2019) to identify differences and make suggestions for optimal culture parameters for stable growth.

Prior to any other tests, the data were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test). The tests used for each analysis can be seen in Table 1. Posthoc tests (Tukey HSD) were conducted where appropriate.

Table 1: Statistical tests applied to the obtained data sets. (*) For the analysis of the antioxidant activity two extremely high values from the 16h-WL treatment were omitted after performing an outlier test (Cook's distance).

Analysis	**Applied statistical method**
Correlation specific growth rate (SGR) vs Light (Fig. 4)	Linear regression
Correlation reproductive area (SGR) vs Area (Fig. 5)	
Correlation O_2 production vs Light (Fig. 10)	
Correlation O_2 production vs Antioxidant activity (Fig. 11)	
Reproductive area within treatments (Fig. 6)	
O_2 production comparison within treatments	Paired t.test
O_2 production comparison on day 1 & 44 (Fig. 8)	Two-Way ANOVA
Reproductive area on the same days (Fig.6)	
Antioxidant activity* (Fig.7)	Two-way ANOVA (type III)
Pigment content	One-Way ANOVA

3. Results

Maximum light intensity of the blue lamps was 40 µmol photons m^{-2} s^{-1}. Due to the low biomass available from the blue light treatments the analysis of the pigment content was restricted to three flasks each, chosen randomly.

3.1 Growth performance

Exclusively positive growth rates were observed in all flask and all treatments over the entire 44 day study period (Fig. 3). The highest specific growth rates (SGR) were found in the 24h white light treatment (24h-WL) reaching as high as 19.7 % d^{-1}. Overall, mean specific growth rates over the entire length of the experiment were much lower. 24h-WL still exhibited the highest average growth rates at 12.6 % d^{-1}, followed by the 16h hour white light treatment (16h-WL) at 10.6 % d^{-1} and 16h blue light (16h-BL) with 4.17 % d^{-1}. The lowest average specific growth rate was observed in the 24h blue light treatment (24h-BL) which only grew by 2.69 % d^{-1}. Both treatments exposed to blue light reached stable SGR after about 24 days. After an initial increase, SGR kept declining in both treatments grown under white light. Both treatments had similar SGRs from day 28 onward (Fig. 3). All calculated SGRs are based on weight measurements.

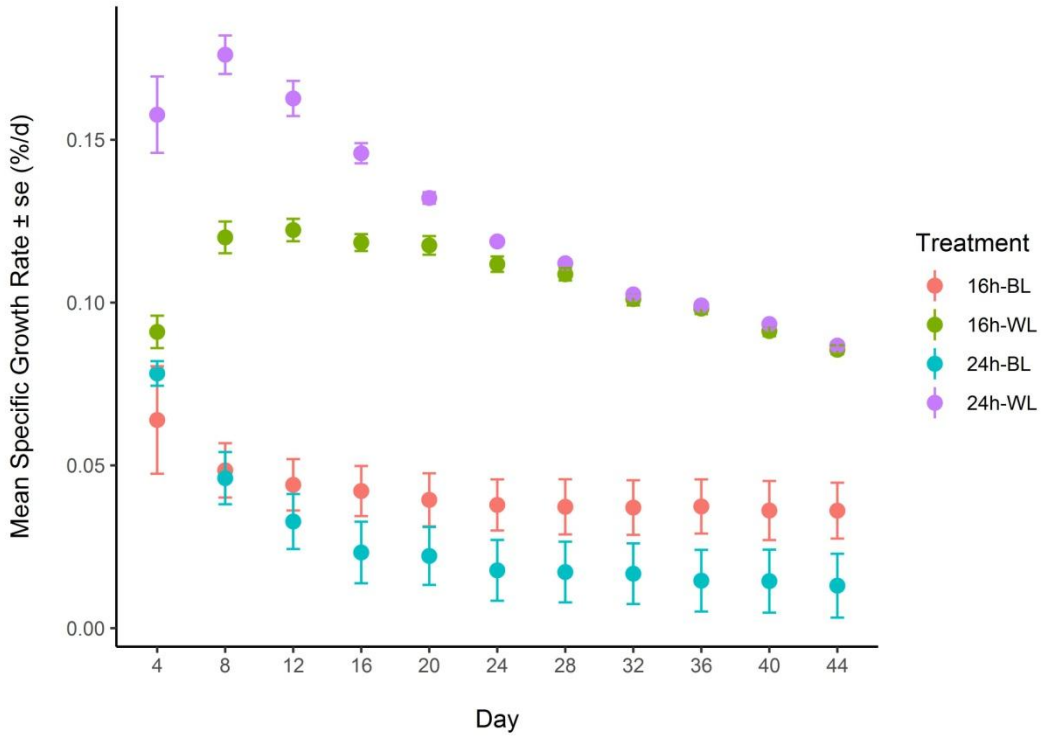

Figure 3: Mean specific growth rates (%/d) over the 44 day duration of the experiment. All calculated SGRs are based on weight measurements. Both blue light treatments reached approximately constant growth rates by day 24. Errorbars indicate standard error; n = 5.

Specific growth rates were significantly correlated with the light intensity of each treatment ($F_{1,218} = 307.3$, $p < 0.001$, $r^2 = 0.58$; $y = 0.0215x - 0.0176$; Fig. 4). Based on this correlation, the highest intensity treatment (24h-WL) had SGRs about three times higher than the treatment with the lowest light intensity (16h-BL).

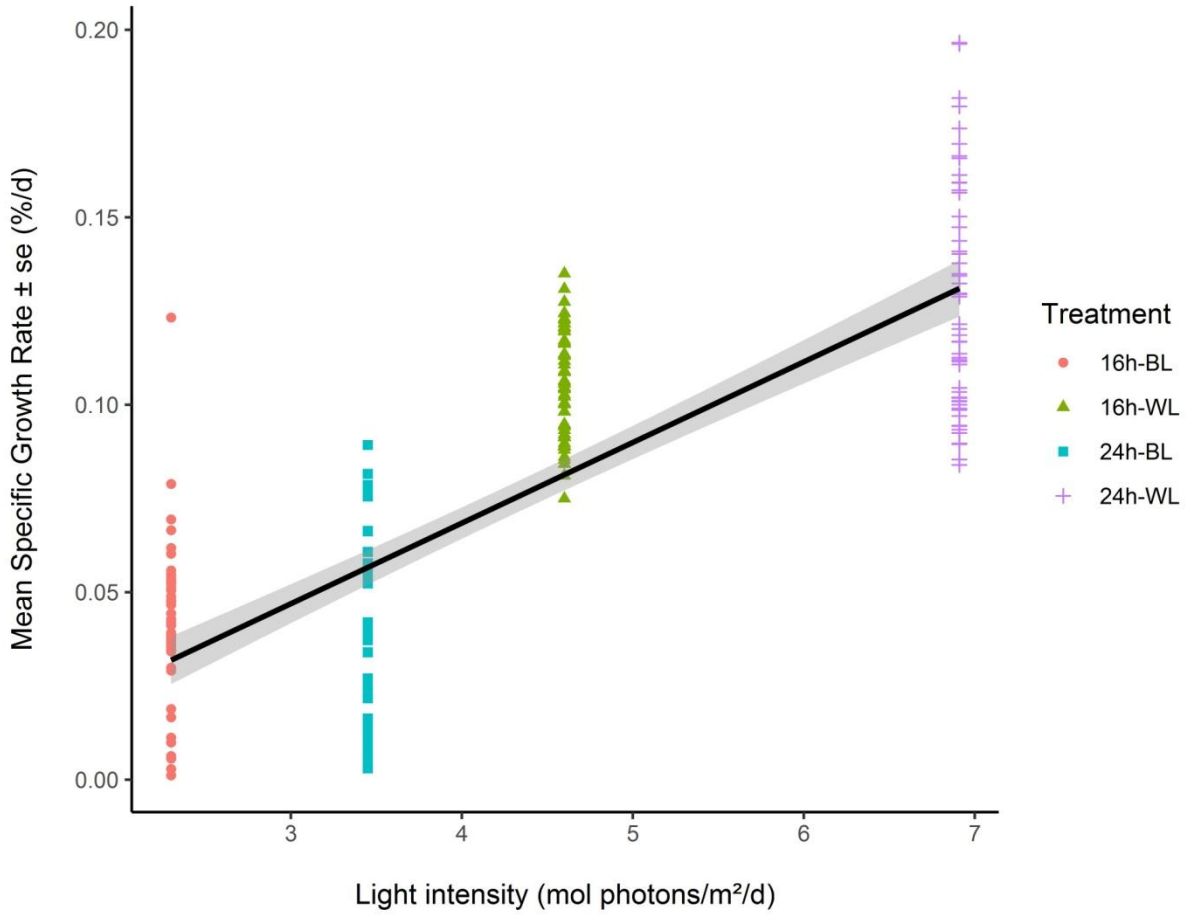

Figure 4: Mean specific growth rates (%/d) corresponding total daily irradiance (mol photons/m^2/d). A significant positive correlation between the two factors was observed ($F_{1,218}$ = 307.3, p < 0.001, r^2 = 0.58; $y = 0.0215x - 0.0176$). n = 55 for each treatment.

3.2 Reproduction

Reproductive edges were identified in the 24h-BL, 16h-WL and 24h-WL treatments (Fig. 5). Most reproduction was observed in the 24h-WL treatment, followed by the 16h-WL treatment. The occurrence of reproduction in the 24h-BL treatment was only a single instance, recorded on day 40 of the experiment. Reproduction in general was minimal, not surpassing 3.5 % of blade area on average and rarely surpassing 2 % of average blade area. Furthermore, reproduction only occurred once the thalli had reached a size of ~600 mm^2. Average reproductive area per fragment per flask was weakly but significantly correlated with average area per fragment per flask ($F_{1,237}$ = 122.7, p < 0.001; $y = 0.00056x - 0.0724$; r^2 = 0.34). Correlations with time (day) and light intensity were considerably weaker (r^2=0.08 and r^2=0.29 respectively). Within any given treatment reproduction did not vary significantly over time (from day 20 onward; no reproduction detected before). With the exception of day 28, the 24h-WL treatment showed significantly higher reproductive activity than both other treatments in which reproduction occurred (Fig. 6).

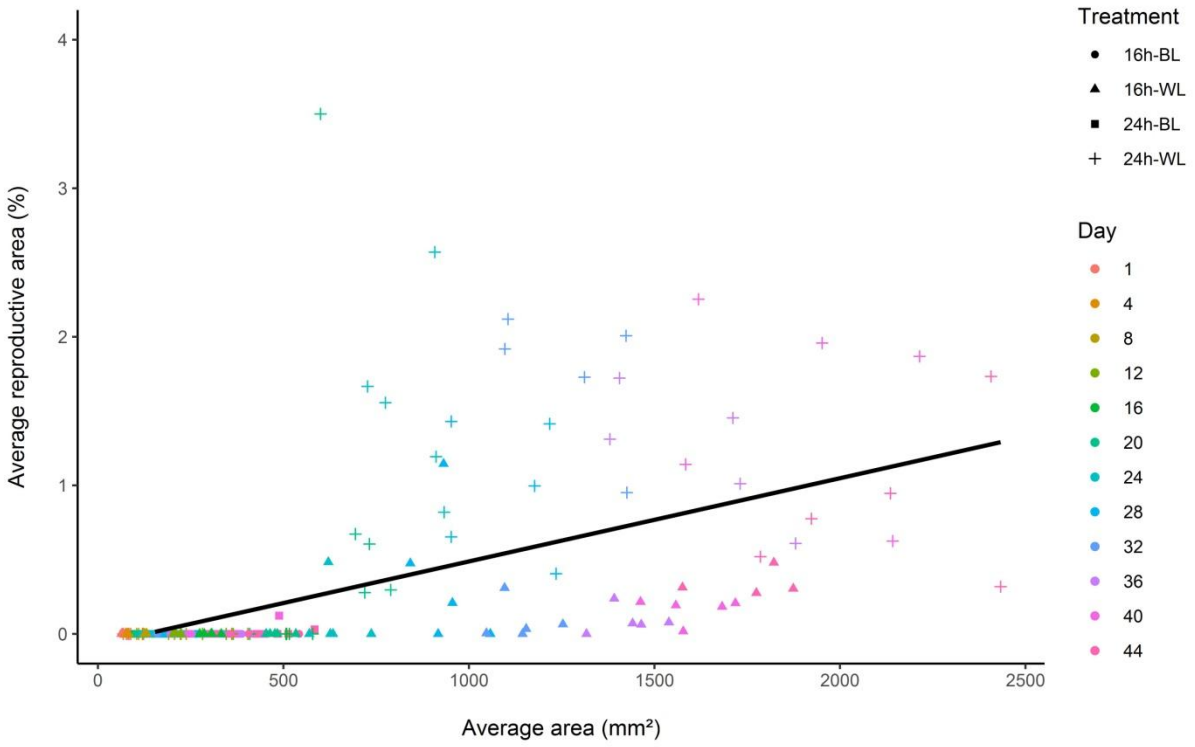

Figure 5: Average reproductive area (%) per fragment within a flask corresponding to average thallus area (mm^2) in the same flask. Different treatments are denoted as different symbols. The measurement day is represented by different colours. No reproduction was observed in the 16h-BL treatment and very little in 24h-BL. The correlation of average reproductive area with average thallus area was significant ($F_{1,237} = 122.7$, $p < 0.001$; $y = 0.00056x - 0.0724$; $r^2 = 0.34$).

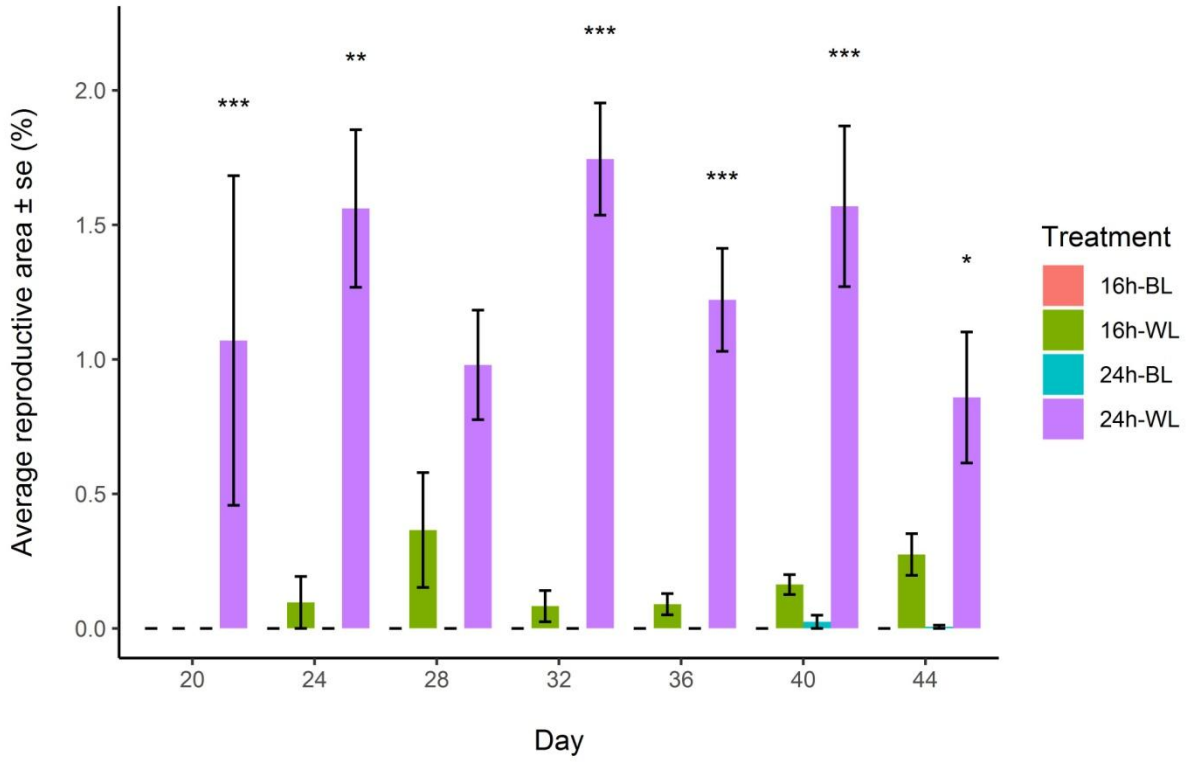

Figure 6: Average reproductive area per treatment over the 44 day period of the experiment. No fragments showed signs of reproduction during the first 16 days of the experiment. With some exceptions in the 24h-BL treatment only the two treatments exposed to white light showed signs of reproduction. n = 5 for each treatment. Error bars represent one standard error. Stars indicate significant differences on the same day (***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$).

3.3 Antioxidant Activity

Light colour significantly affected antioxidant activity in the treatments ($F_{1,14} = 6.1768$, $p \approx 0.026$). Neither daylength ($p \approx 0.062$) nor the interaction term ($p \approx 0.598$) were significant. No treatment showed significantly higher antioxidant activity than the wild collected control sample (Fig. 7). The highest level of antioxidant activity in the tested treatments was observed in 24h-BL (44.96 µmol Trolox equivalent/g dry alga extract; Fig. 7). However, the wild collected control sample showed a higher activity than this (47.87 µmol Trolox equivalent/g dry alga extract; Fig. 7). The 24h-BL treatment had consistently higher values than both the 16h-WL treatment and the 24h-WL treatment.

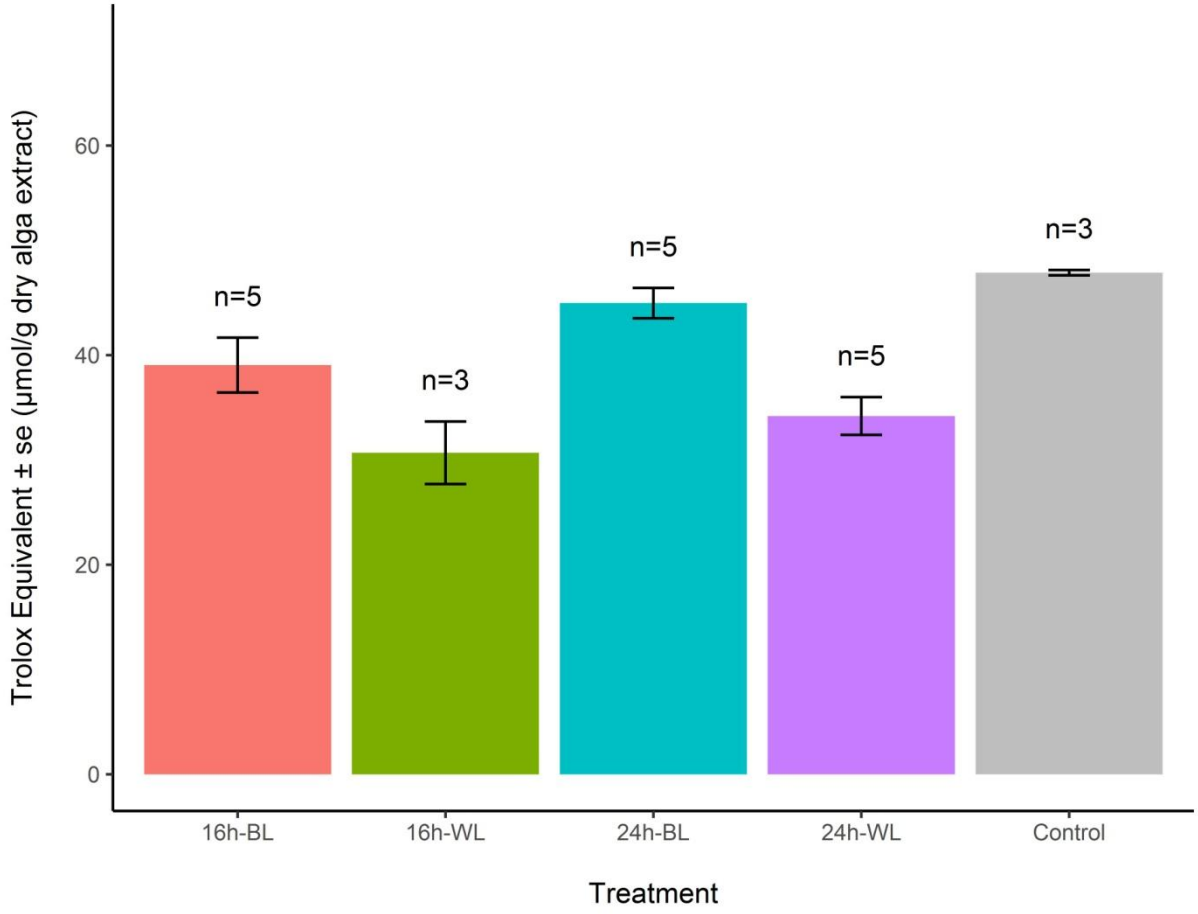

Figure 7: Antioxidant activity measured as µmol Trolox Equivalent per gram dry algae extract. Light colour was the only significant factor ($F_{1,14} = 6.1768$, $p \approx 0.026$). Error bars represent plus/minus one standard error. Replicates are given beneath letters of significance. Two values were omitted from the 16h-WL treatment after performing outlier tests.

3.4 Pigment Content

All treatments had significantly lower carotenoid content than the wild collected control sample ($F_{4,10}$ = 12.93, p < 0.001; Fig. 8). Chlorophyll content was considerably lower in the 16h-WL treatment than in all other treatments ($F_{4,10}$ = 16.03, p < 0.001). All other treatments did not differ significantly from each other. The control had intermediate values. This trend is largely the same when looking at chlorophyll a and b content individually. However, the control had similar chlorophyll a content to both blue and the 24h-WL treatments. With the exception of the 24h-WL treatment Chlorophyll content was inversely related to total daily irradiance. The same observation was made for the carotenoid content.

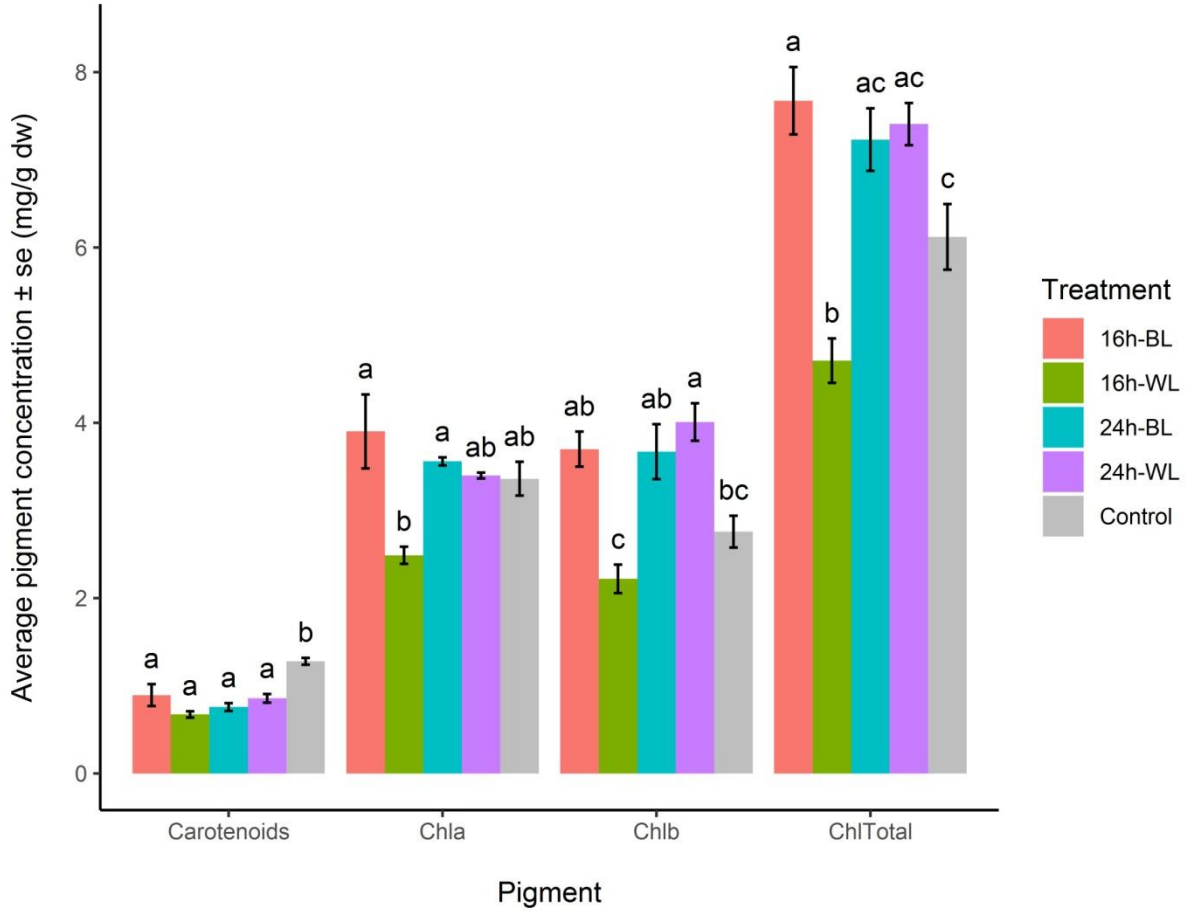

Figure 8: Average concentration of carotenoids, chlorophyll a (Chla), chlorophyll b (Chlb) and total chlorophyll (ChlTotal) in mg/g dry weight. The treatment with the lowest light intensity (16h-BL) had the highest chlorophyll content. Letters show significant differences in pigment content between treatments. Error bars indicate standard error. n = 3 for all treatments and 2 for the wild control.

3.5 Oxygen production

All treatments showed a marked decline in oxygen production over the study period (Fig. 9). This decline in O_2 production, between day one and day 44, was significant for all treatments (24h-BL: $t(4) = 5.7287$, $p = 0.004597$; 24h-WL: $t(4) = 6.7434$, $p = 0.002521$; 16h-BL: $t(4) = 6.8333$, $p = 0.002399$; 16h-WL: $t(4) = 7.8745$, $p = 0.001406$). Oxygen production on day 44 was reduced by approximately 60% in both 24h treatments and by approximately 70% in both 16h treatments (Fig. 8). However, this difference in decline was not statistically significant.

Treatments showed statistically significant differences in oxygen production on day one of the experiment (Fig 9A). However, the interaction term was not significant ($F_{1,16} = 38.085$, $p \approx 0.477$) and only light colour was a significant factor. Daylength, as expected, had no effect since the treatments had just been set up ($p = 0.962$). While the 24h-WL treatment produced 0.0242 µmol O_2/g/s (0.0258 for 16h-WL) the blue light treatments only amounted to 0.013 and 0.0116 µmol O_2/g/s for 24h and 16h illumination, respectively.

Treatments also showed statistically significant differences in oxygen production on day 44 of the experiment (Fig 9B). Just as on day one the interaction term was not significant ($F_{1,16} = 20.045$, $p \approx 0.834$). Light colour, again, was a significant factor ($p < 0.001$) contributing to the differences between treatments. Unlike on day one however, daylength was also significant ($p \approx 0.032$). The 24h-WL treatment had the highest oxygen production rates (0.0098 µmol O_2/g/s) among all treatments, especially higher than the blue light treatments. Both of the latter produced on average 0.0054 (24h-BL) and 0.0034 µmol O_2/g/s (16h-BL)**.**

Oxygen production also showed a significant correlation with light intensity of the treatments (Fig. 10) both on day one ($F_{1,18} = 15.94$, $p < 0.001$, $r^2 = 0.47$, $y = 0.0031x + 0.0054$) and on day 44 of the experiment ($F_{1,18} = 27.35$, $p < 0.001$, $r^2 = 0.60$, $y = 0.0014x + 0.00055$). Correlation was weaker on day one. This is in line with the fact that treatments with the same light colour did not show significant differences (Fig. 9A).

Antioxidant activity showed a significant negative correlation with oxygen production ($F_{1,16} = 6.111$, $p \approx 0.025$, $r^2 = 0.28$; $y = -1086.03x + 45.009$; Fig. 11).

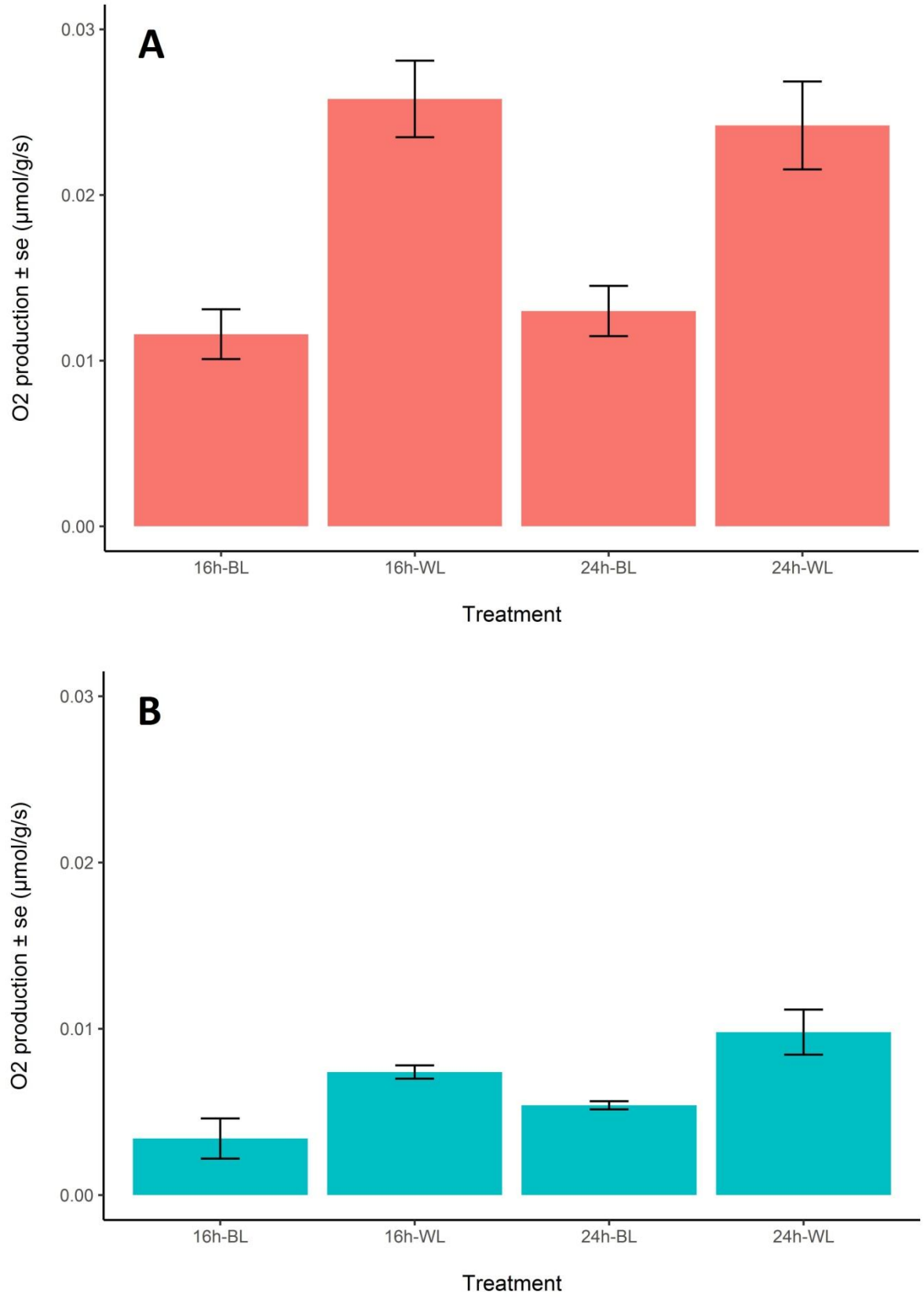

Figure 9: (A): Mean oxygen production rates (µmol/g/s algae) for each treatment on day one of the experiment. Light colour was the only significant factor ($F_{1,16}$ = 38.085, $p < 0.001$). **(B):** Oxygen production rates (µmol/g/s algae) for each treatment on day 44 of the experiment. The interaction between light colour and daylength was not significant ($F_{1,16}$ = 20.045, $p \approx 0.834$) but both factors individually were ($p < 0.001$ and $p \approx 0.032$ for light colour and daylength, respectively). Error bars indicate one standard error. The number of replicates was 5 for each treatment.

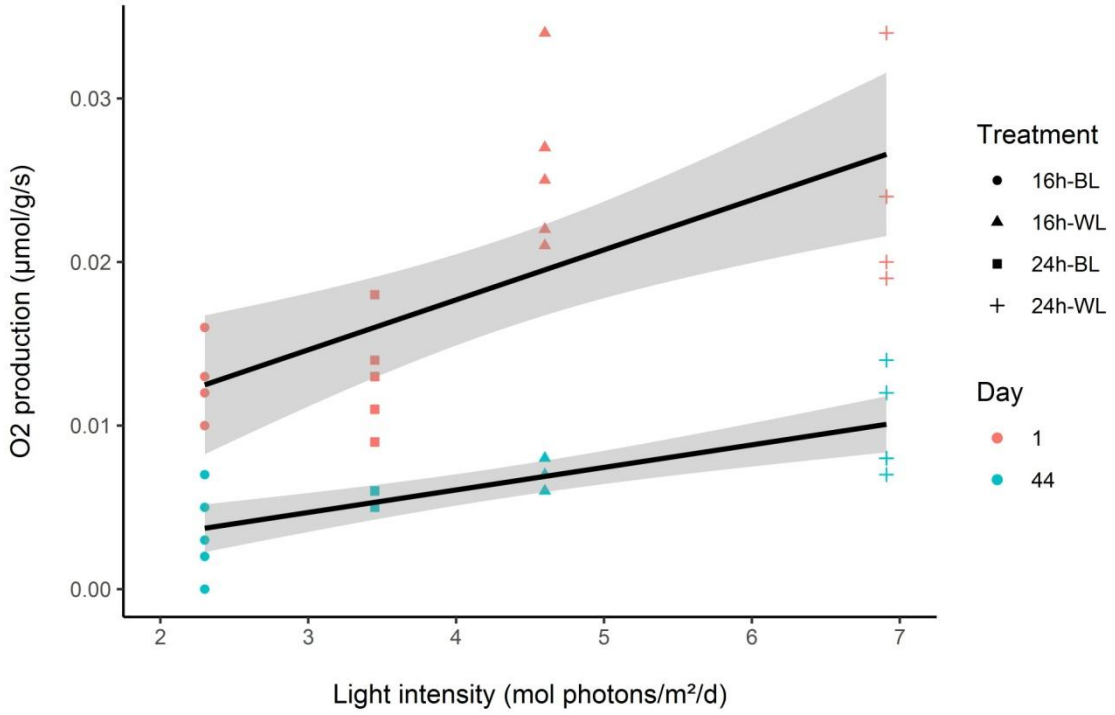

Figure 10: The oxygen production (µmol/g/s) of all four treatments corresponding to the total daily irradiance (mol photons/m^2/d) of each treatment. Oxygen production was significantly correlated with light intensity both on day one ($F_{1,18}$=15.94, p < 0.001, r^2=0.47, $y = 0.0031x + 0.0054$) and on day 44 of the experiment ($F_{1,18}$=27.35, p < 0.001, r^2=0.60, $y = 0.0014x + 0.00055$). Dashed lines indicate the 95% confidence interval. The number of replicates for each treatment on each day was 5.

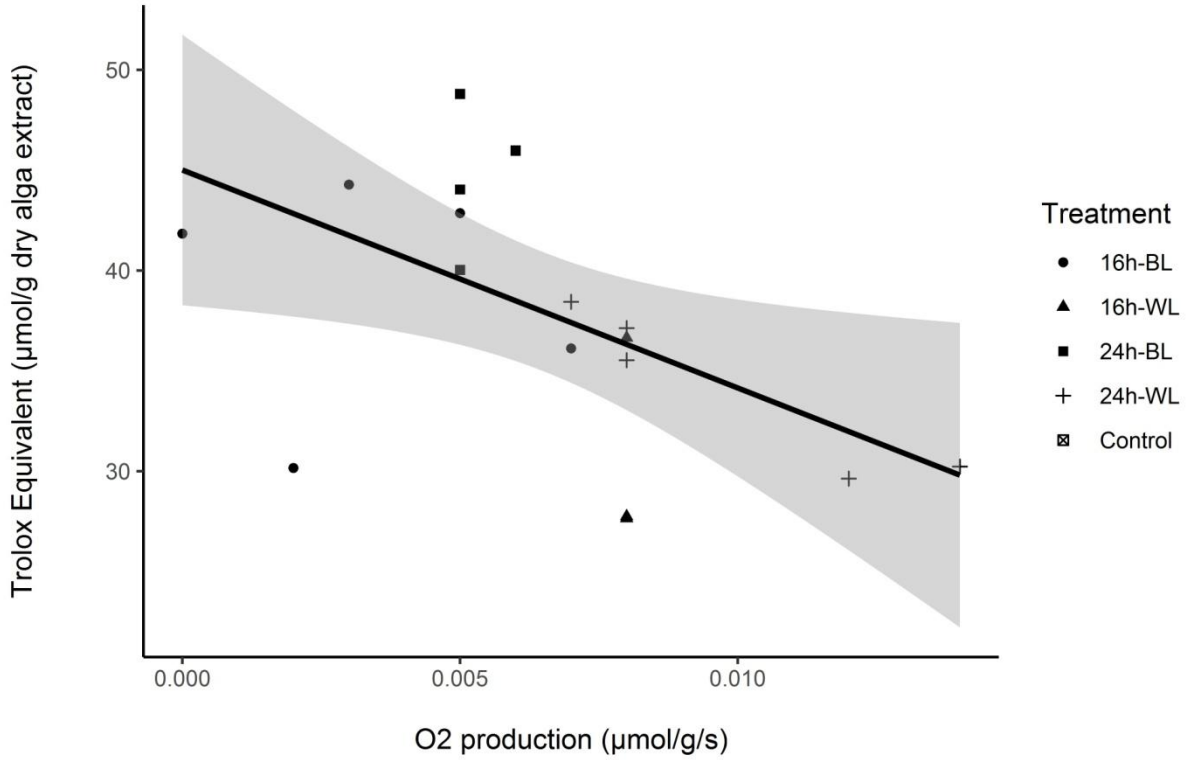

Figure 11: Relationship between antioxidant activity and oxygen production. A significant negative correlation was observed ($F_{1,16}$ = 6.111, p ≈ 0.025, r^2 = 0.28; $y = -1086.03x + 45.009$). The shaded are indicates the 95% confidence intervals.

4. Discussion

4.1 Growth

While the 24h-WL treatment did show the highest growth rates at 17.6 % d^{-1}, the 16h-WL treatment had far lower growth rates at 12 % d^{-1}, even though it was grown under supposedly optimum conditions (Fortes and Lüning, 1980). Fortes and Lüning (1980) reported that growth saturation occurred at above 70 µmol photons/m^2/s in *Uva lactuca* collected on Helgoland. In their study they furthermore measured the highest growth rates for daylengths of 16-24 hours (above 20 % d^{-1} for each). The highest growth rates observed in the present study coincide with values commonly reported in the literature. Several studies report growth rates between 10 and 20 % d^{-1} (Bruhn et al., 2011; Neori et al., 1991; Taylor et al., 2001), the first two for *Ulva lactuca*, the last for *Ulva rigida* & *U. curvata*. Lapointe and Tenore (1981) measured an SGR of 36 % d^{-1} for *Ulva fasciata*. However, they worked with added nitrogen specifically to enhance growth. While the peak SGRs fell into the upper part of this range, most treatments had average growth rates closer to 10 % d^{-1} or in case of the blue light treatments well below that. This points to the fact that the collected algae had different optimum conditions than expected or that one of the other culture parameters had a growth limiting effect.

The fact that the growth rate in both white light treatments kept declining makes limitation probable, be it either from nutrient, light or otherwise. Since the decline in SGR occurred after an initial increase during the first eight days it is likely that SGR declined due to changes in culture parameters resulting from a higher biomass in the flasks. Photosynthetic saturation occurs at much higher irradiances than saturation of growth. According to Yokohama (1973) and Fortes and Lüning, (1980) photosynthesis in *Ulva pertusa* is saturated at 200 µmol photons/m^2/s. Assuming the thalli in this study were not nutrient limited (at least in the beginning of the experiment) would allow to conclude that those thalli grown under white light should have achieved their maximum growth rates, but nowhere near their maximum photosynthetic rates. *Ulva* in general seems to be very shade tolerant. Studies by Riccardi and Solidoro, and Sand-Jensen observed growth for light intensities as low as 1.7 and 1.9 µmol photons/m^2/s (Sand-Jensen, 1988; Riccardi and Solidoro, 1996). This adaptation is likely one of the factors that allows *Ulva* to form "green tides" since they are able to grow even under excessive self shading. The declining growth rates in the white light treatments could also be, at least in part, a consequence of reproduction occurring in these two treatments. While evidence for trade-offs between growth and reproduction in seaweeds are limited, several studies have found evidence for the inverse relationship between vegetative growth and reproduction hypothesised by De Wreede and Klinger (1988; Chu et al., 2011; Guillemin et al., 2014; Graham, 2002).

An apparently pronounced effect of light colour on SGR could be observed in this experiment. This is likely in large part due to the fact that the intensity of blue light was only half that of white light. However, in the literature several examples for at least average growth rates under blue light exist. Le et al. (2018) measured SGRs of ~12 %/d under blue LEDs as well as white fluorescent light with a 12/12 light/dark cycle and 100 µmol photons/m^2/s.

Other studies have pointed to the importance of UV radiation for growth and photosynthesis (Altamirano et al., 2000). It affects pigment content, growth rate and photosynthetic performance in *Ulva rigida*. The removal of UVB in particular enhanced photosynthesis. However, treatments

subjected solely to photosynthetically active radiation (PAR) always grew worse than any treatment including UV (Altamirano et al., 2000)**.** Keeping this in mind the algae in this experiment are not likely to have reached their maximum growth rates under the experimental conditions.

4.2 Oxygen production

Similar to the trend observed in SGR, oxygen production at the end of the experiment was also much lower than in the beginning. Assuming a similar saturation point of photosynthesis for *Ulva* sp. in the present study as that reported by Yokohama (1973) for *Ulva pertusa* (200 µmol photons/m^2/s), the decreasing oxygen production rates are not attributable to a light stress factor, at least for the white light treatments. Riccardi and Solidoro (1996) reported only slight photosynthetic inhibition for light intensities up to 2280µmol photons/m^2/s of *Ulva rigida* in Venice lagoon. While local and species specific adaptations are known to lead to varying tolerance levels, this illustrates that *Ulva* in general is very light tolerant. A broad range of tolerance seems logical, especially when viewed in the context of the intertidal as the natural habitat of *Ulva.* In general, oxygen production values obtained from the literature are considerably higher (3-10x) than the values measured at the beginning of this experiment. At comparable light intensities to this study, Riccardi and Solidoro (1996) measured 0.104 µmol/g/s for *Ulva rigida*. 0.05-0.111 µmol/g/s have been measured in *Ulva lactuca* (Lartigue et al., 2003). However, the latter was measured in conjunction with fluctuating salinities, slightly higher irradiance (200 µmol photons/m^2/s), and under considerably warmer conditions (28 °C). Other studies have reported similar values to the ones obtained in this study under much higher light intensities, for example 0.03 µmol/g/s at 700 µmol photons (Collén et al., 1995).

Potential reasons for the decline in oxygen production are nutrient limitations that occurred especially in well growing treatments under white light. It is also possible that during the initial measurement the collected specimens were still affected by some parameter they had been exposed to in the field. Another explanation could be found in carbon uptake inhibition as a limiting factor for photosynthesis and thus O_2 production. *Ulva rigida*, for example, is known to have both extracellular and intracellular carbon anhydrases that could be affected by changes in water chemistry and pH (Björk et al., 1992). Light seems unlikely to be the sole deciding factor since the decline in oxygen production was approximately equal in all treatments, even though the light intensity and colour differed. Micronutrients might have been missing in the artificial seawater, but algae had sufficient stores left on day one and were able to mitigate this. In a follow-up study the effect of these micronutrients on growth, oxygen production, reproduction and antioxidant content/polysaccharide content could be studied. This would grant insight in the commercial feasibility of different culture media. Carefully engineered media might have higher biomass yields but are also more expensive and time consuming to produce, especially on large scales required for industrial production.

4.3 Reproduction

The results for reproduction are somewhat obscured by the fact that blue light treatments in general received less light and thus grew less. Only treatments under white light showed reproduction (with two exceptions in the 24h-BL treatment). Whether the light colour or whether thallus size is the deciding factor here cannot be said for certain. Based on a comparison between the correlation of relative reproductive area and average thallus size, thallus size seems to be the more important factor. This correlates well with other studies on size dependent reproduction in macroalgae. Zou et al. (2006) found threshold sizes after which reproduction occured in *Hiziaka fusiformes*. A clear

correlation of reproductive output with increasing size has been reported for various fucoid algae (Mathieson and Guo, 1992).

However, light quality is also known to affect reproduction in marine macroalgae. In studies on *Ulva lactuca* and *Laminaria* it was found that exposure to blue light could prevent gamete release (Lüning, 1981; Wichard and Oertel, 2010). While the results in this study do not contradict those findings, they cannot confirm them either. Reproduction under blue light did only occur in one instance during the entire duration of the experiment; however, those treatments also exhibited the lowest growth rates and thus did not reach the size of the fragments in the white light treatments that became reproductive. To disentangle these factors (thallus size and light colour) a further experiment is needed. Special attention should be given to the light intensity to make sure that both treatments experience the same. The prevention of gamete release under continuous light that was observed by Lüning et al. (2008) in *Ulva pseudocurvata* could not be seen in the present study. Gametes were released frequently as evident from the transparent edges of many fragments. As demonstrated in the same study moonlight also seems to play an important role in modulating the reproductive cycles in *Ulva* (Lüning et al., 2008). For future studies this provides another factor to consider when developing systems for the cultivation of *Ulva* with minimal reproduction.

4.4 Antioxidants

Antioxidant activity followed the expected pattern, with blue light treatments showing higher activity than their white light counterparts. Again, as expected, among the algae grown under blue light those in the 24h-BL treatment showed the highest antioxidant activity. This is in line with several other studies (Le et al., 2018) and points towards the importance of blue light in regulating internal biochemical processes in algae. Blue light has also been identified as an important regulator in seaweed photosynthesis and has been shown to induce chlorophyll synthesis in *Ulva rigida* (Lopez-Figueroa and Niell, 1989). It is also important to note that the treatment with the lower light intensity showed the highest antioxidant activity. If the desired aim of a culture is to optimise antioxidant content of *Ulva* sp., a middle ground between high growth rate under high light and high antioxidant content under low irradiances might be appropriate. To determine this further studies on the importance of light intensity relative to light colour are needed. The values for antioxidant activity for *Ulva* sp. obtained in this study are relatively high compared to other algae (Table 2). If confirmed by further research *Ulva* sp. could prove to be a valuable and efficient source of antioxidants.

Other studies have reported similar findings for enhanced polysaccharide yield under blue light in other algae (Carmona *et al.*, 1998; Singh and Das, 2011). An enhanced polysaccharide content in *Ulva* would certainly mean higher ulvan content. Since the ulvans are known for their potent antioxidant activity (Qi *et al.*, 2005; Qi and Sun, 2015; Qi *et al.*, 2010; Le *et al.*, 2018), this is a potential explanation for the observed increased antioxidant activity under blue light in the present study. However, the exact mechanisms by which light quality affects theses processes are still unknown.

A possible mechanism that could explain higher antioxidant activity under blue light is the degree of sulfation of molecules that make up ulvan. However, it is yet unclear whether a higher sulfate content has a positive (Qi *et al.*, 2005; Qi and Sun, 2015) or negative (Le *et al.*, 2018; Shao *et al.*, 2013) effect on antioxidant activity, but all studies reported changes in ulvan sulfation under blue light.

It is important to keep in mind that the algae grown under blue light showed significantly lower specific growth rates (SGR) than those grown under white light. While this is certainly attributable to the fact that the blue light treatments irradiance was a lot lower (40 compared to 80µmol photons/m^2/s) potentially lower growth rates are an important trade-off to consider when thinking about *Ulva* cultivation in a commercial context. A follow-up study focussing on blue light with higher intensity could shed some light on the extent of this trade-off. Furthermore, while the 24h-BL treatment did show the highest antioxidant activity among the algae grown in the lab, it should also not be forgotten that the wild collected field control did exhibit even higher activity.

Table 2: Selected antioxidant activities for algae, fruit and vegetables from the literature. Values expressed as µmol Trolox Equivalent (TE) per fresh weight (fw), dry weight (dw) or dry extract (de). [a](Cofrades et al., 2010), [b](Álvarez-Gómez, Korbee and Figueroa, 2016) , [c](Magnusson et al., 2015), [d](Nilsson et al., 2005).

Species	**Antioxidant activity (by ABTS)**
Himanthalia elongata	14.05 µmol TE g^{-1} fw[a]
Undaria pinnatifida	1.81 µmol TE g^{-1} fw[a]
Porphyra umbilicalis	0.01 µmol TE g^{-1} fw[a] 5.8-7.8 µmol TE g^{-1} dw[b]
Derbesia tenussima	~5.89 µmol TE g^{-1} de[c]
Ulva sp.	~4.75-9.21 µmol TE g^{-1} dw ~27.64-48.7 µmol TE g^{-1} de
Orange	37.4 µmol TE g^{-1} fw[d]
Spinach (concentrate)	92.4 µmol TE g^{-1} fw[d]
Spinach (fresh)	2 µmol TE g^{-1} fw[d]
Carrot (concentrate)	17.2 µmol TE g^{-1} fw[d]
Carrot (fresh)	0.34 µmol TE g^{-1} fw[d]

Nevertheless, one does have to recognise that the antioxidant content of wild grown algae is most likely subject to seasonal changes. Since antioxidants are produced at a higher rate when the individual is under stress, the content might even be weather dependent. Salinity stress has also been shown to affect antioxidant content in *Ulva fasciata* (Lu et al., 2006). Short term hypersaline conditions, for example during low tide, are thus likely to affect the antioxidant content of wild harvested material. Wild collection is also not always an option, especially if a constant amount of material is needed all year round, as would most likely be the case for algae used for packaging. Depending on the species and the desired amount, the sustainability of the entire endeavour also has to be considered.

When Ulva is meant to be specifically cultured for its high antioxidant activity, there are a couple of other parameters at the farmer's disposal. Zhong et al. (2015) demonstrated that addition of <750mg/L Selenium into cultures increases growth rate and antioxidant content of *Ulva fasciata*. Other micronutrients such as iron, copper and manganese are already known to be essential for photosynthesis and growth (Raven, 1988; Martin and Fitzwater, 1988; Falkowski and Raven, 2013). As mentioned above, salinity stress is also a suitable tool to manipulate antioxidant content *in vivo*. Along the same line Altamirano et al. (2000) demonstrated that UVB had positive effects on pigment content. It is likely that this directly translated into higher antioxidant activity.

4.5 Pigments

In general, pigmentation in algae and higher plants is inversely related to increasing irradiance (Anderson, 1986; Henley and Ramus, 1989). In the present study this holds true, since the treatment

under the lowest light intensity had the higher chlorophyll content than the wild sample. The treatment that most closely resembled natural conditions (16h-WL) had the lowest chlorophyll content. However, the 24h-Wl treatment showed equal chlorophyll content to both blue light treatments even though it was subjected to the highest irradiances. The previously discussed importance of blue light in chlorophyll synthesis would explain the high concentrations observed in the blue light treatments, but fails to provide an explanation for the observations in the 24h-WL treatment. It has been suggested that chlorophyll complexes can act as nitrogen storage in green algae (Gevaert and Rees, 2015). This could at least partially explain the high chlorophyll content under 24h-WL.

The 24h-WL treatment was consistently at odds with trends observed in this experiment and commonly reported in the literature (more light - less pigments; less light - higher chl to carotenoid ratio). Numerous studies have demonstrated this relationship both in higher plants as well as seaweed (Ramus, 1983; Anderson, 1986) and *Ulva* in particular. After a week of cultivation at 250 µmol photons/m^2/s, *Ulva lactuca* thalli had less than half the chlorophyll content of thalli cultured at 30 µmol photons/m^2/s (Fortes and Lüning, 1980). It is thus likely that the 24h-Wl treatment was affected by another factor the other treatments did not experience. It is possible that the increased stocking density in the 24h-WL treatment led to pronounced shading, resulting in similar light levels to the blue light treatments. However, the 16h-WL treatment did not have that much less biomass, so shading is unlikely to be the sole factor at play here. Light colour seems unlikely to be the culprit. Under light limiting conditions, light colour has been reported to not influence pigment content and composition (Ramus, 1983).

Carotenoids are known for playing a role in photoprotection (Siefermann-Harms, 1985). The decreasing Chl/Carotenoid ratio is explained by that, as well as the slight trend for higher intensity treatments to contain more carotenoids. This especially explains the significantly higher carotenoid content in the control exposed to natural light intensities which tended to be much higher than in the treatments.

4.6 Outlook

For future studies, the focus should be on using same light intensity for different light colours. To optimise growth, mixed treatments with partial blue and white light might be interesting. Le et al. (2018) tested a similar approach including red light and did not find any significant effects on growth or antioxidant activity. Keeping stocking density the same during the experiment would also allow for an easier attribution of observations to individual experimental factors. It has been shown, for example, that stocking density can significantly influence antioxidant content (Magnusson et al., 2015). To optimise the reproductive pattern of *Ulva*, more studies on the underlying biochemical processes would be advantageous. Especially the swarming inhibitors identified in *Ulva* could prove to be a powerful tool for the control of reproduction in culture (Nilsen and Nordby, 1975; Stratmann et al., 1996; Wichard and Oertel, 2010; Vesty et al., 2015). Hitherto none of these sporulation and swarming inhibitors have been chemically characterised.

Interesting other factors worth to consider include the bacterial symbionts associated with *Ulva*. They seem to play an important role in the morphological development of *Ulva* (Wichard and Oertel, 2010). It has been demonstrated that *Ulva lactuca* grown in axcenic conditions developed a “pin-cushion” like growth form rather than the usual sheet-like fashion (Provasoli and Pintner, 1980).

Similar results have been reported for other species of *Ulva* (Nakanishi et al., 1996; Nakanishi et al., 1999), and Spoerner et al. (2012) even managed to identify specific bacteria and compounds that were necessary for the development of *Ulva mutabilis* gametes. As this demonstrates the importance of the microbiome of the algae, the question has to be asked what other processes are catalysed by associated microorganisms. Especially for marine invertebrates such as sponges or ascidians it has been found that the associated microorganisms rather than the macroscopic host are the source for valuable bioactive compounds with medical applications (Newman and Hill, 2006; Tianero et al., 2015). Villareal-Gómez et al. (2010) demonstrated that extracts produced from several seaweeds as well as their associated microbiota showed anticancer and antibacterial activity. It might be worthwhile to look more deeply into the microbiology of seaweed-associated microbes, especially in the search for novel bioactive compounds.

4.7 Conclusion

In conclusion, the results obtained in the present study do not provide a concrete answer as to the effectiveness of daylength or light colour adjustment in preventing reproduction in *Ulva* sp. However, even though most of the results are obscured by the fact that the light intensity was not uniform between the treatments a few key points can nonetheless be made:

1. Blue light allows for increased antioxidant content even at low light intensity. This shows that light colour can be a powerful tool to adjust seaweed chemistry based on the desired culture outcome.
2. Reproduction seems to be at least partially size dependent. Identifying appropriate times for harvesting is thus a key in preventing biomass loss through reproduction. The importance of light colour in regulating reproduction in *Ulva* sp. warrants further investigation.

5. Acknowledgements

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