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From diatom to a sustainable business: optimizing the fucoxanthin production by varying light intensities and temperature

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Abstract

Phaeodactylum tricornutum is a robust and widely studied diatom with a potential usage in the commercial algae cultivation because of its reportedly high fucoxanthin content. A lot of research is going into fucoxanthin since it has a possible medicinal potential. Therefore, knowing the factors which have an impact on the fucoxanthin production by *P. tricornutum* may increase the interest in commercial usage and stimulate more research into its application. The effects of irradiance on the fucoxanthin production are quite well known, but the effects of temperature are understudied. In the present study, the effects of different irradiances and temperatures on the fucoxanthin production of *P. tricornutum* were determined. The results of these experiments were used to setup a cultivation system where the fucoxanthin content could passively increase without adding extra nutrients. At high irradiances *P. tricornutum* showed lower fucoxanthin levels compared to low irradiances. Higher temperature also has a negative effect on the fucoxanthin content, but only in higher irradiances. The designed cultivation setup showed that the passive increase of fucoxanthin content worked, but it was not yet efficient enough to outcompete the growth of a second batch in the same time frame. The cultivation system still has a lot of room for improvement which may lead to greater efficiency. Overall, this study provides a good starting point for research into the effects of temperature on the fucoxanthin production and may lead to a better optimized cultivation setup.

Contents

Introduction

For millions of years primary producers in the oceans have produced oxygen and sequestered carbon dioxide. In recent years the interest in marine algae raised because they could be used as a source of biomass and biomolecules (Bozarth et al. 2009). An interesting group of the marine algae are the diatoms. Diatoms are a diverse group of algae with species living in fresh and marine waters. These photosynthetically active organisms produce an extra silicate housing around their cells. Estimations are that diatoms can account for 40% of the marine primary production (Falkowski et al. 1998; Sarthou et al. 2005). They are also major players in the biochemical cycling of nutrients (Nitrogen, Phosphorus, Silicate and Iron) and carbon fluxes (Buesseler 1998). The photosynthetic complex of diatoms consist of chlorophyll a, chlorophyll c and fucoxanthin which enables them to capture light in the blue/green area (Katoh et al. 1989). Overall diatoms are very robust, are capable of growing in almost every photic zone and even grow under sea ice and react to sea ice freezing (Janech et al. 2006).

Due to the their robustness and flexibility diatoms have become an interesting organism for the use in biotechnology (Bozarth et al. 2009). At the same time they also have an enormous economic potential, because they contain bioactive compounds which could be used for creating jet fuel to cosmetic chemicals (Bozarth et al. 2009). Especially the carotenoid fucoxanthin and all its derivatives gained special attention from the scientific community (Muradian et al. 2015). Fucoxanthin has been studied wildly and a lot of possible applications have been found, especially as a compound of medicines against certain deceases. Fucoxanthin is argued to have an anti-obesity and anti-diabetic effect (Maeda et al. 2009), helping in preventing cardiovascular diseases (Riccioni et al. 2011) and having an anti-cancer effect (Kumar et al. 2013). The effects of fucoxanthin has recently been reviewed (Zhang et al. 2015; Muradian et al. 2015) which shows that fucoxanthin may indeed have these effects in animal models but clinical trials in humans are scarce. A problem of fucoxanthin is that it is an unstable compound (Zhang et al. 2015), but the reported side effects are minimal and a lot of research is going into the stability of fucoxanthin (Zhang et al. 2015; Muradian et al. 2015).

Fucoxanthin is now mainly harvested from brown seaweeds grown in Asia but it has been reported that the production in the diatom *Phaeodactylum tricornutum* is ten times higher (Kim et al. 2012). *P. tricornutum* is also interesting because its genome is small, less than 20Mb, and well-studied (Scala et al. 2002; Leu & Boussiba 2014). All these properties make *P. tricornutum* an interesting model species for the production of fucoxanthin on a commercial basis.

Effect of irradiance on fucoxanthin content

In the study of MacIntyre & Geider (1996) it has been shown that algae can alter the amount and composition of their pigments for optimal light harvesting. For fucoxanthin it has been shown that the total amount of fucoxanthin decreases with increasing light intensity (Laviale & Neveux 2011). In the study of Gómez-Loredo et al. (2016) *P. tricornutum* was grown under different light conditions, ranging from 9.1 to 62.0 µmoll photons m^{-2} s^{-1}. Under aerated conditions *P. tricornutum* showed the highest fucoxanthin concentration at 13.5 µmoll photons m^{-2} s^{-1}. However the light intensity of 13.5 µmoll photons m^{-2} s^{-1} did not show the highest growth rate and maximum cell density, this was observed in 62.0 µmoll photons m^{-2} s^{-1}. When growing *P. tricornutum* for the production of fucoxanthin on a commercial basis the results of these studies must be taken into account. This implies a growth regime where *P. tricornutum* first will be grown under optimal light conditions to reach the highest maximum cell density at the fastest rate. When the maximum cell density is reached, *P. tricornutum* should then be placed under the optimal light intensity for the highest concentration of fucoxanthin.

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Temperature effects

It is known that temperature has an effect on the growth rate of marine diatoms (Montagnes & Franklin 2001; Raven & Geider 1988). Montagnes and Franklin (2001) showed in their study an increasing growth rate with increasing temperature (9°-25°Celsius) until a certain optimum, after that optimum the growth rate declined. Every species has its own optimum temperature, 20°Celsius (C) was the optimum temperature for the maximum growth rate of *P. tricornutum*. Temperature also has a wide range of effects on the photosynthetic capabilities of marine algae (Davidson 1991), but no studies could be found on the effects of temperature on pigment composition in marine algae. However, increasing temperatures could be an extra stress factor on the growth of *P. tricornutum* which may lead to different amounts of pigments under different light conditions. Also, higher temperature may reduce the amount of energy needed for the photosynthesis which could reduce the amount of light harvesting pigments. Since fucoxanthin is a primary part of the photosystem in *P. tricornutum*, it can be argued that the amount of fucoxanthin may vary under higher temperatures under different light conditions.

Aim of the study

The aim of this study was to optimize fucoxanthin production in *P. tricornutum*. Two factors were taken into account for the optimization, in particular irradiance and temperature. The effect of different light intensities at two temperatures on the production of fucoxanthin was studied to determine the optimal growth rate and optimal fucoxanthin production. The results of these experiments were then used to setup an experiment which in theory would have the highest amount of biomass with the highest fucoxanthin content in the shortest time period.

Application

The results from this study could have implications for commercial algae cultivation. Insights could be gained on the effects of irradiance and temperature on the pigment composition of *P. tricornutum*. Some pigments have an interesting commercial value, all the more reason for the optimization of the algae cultivation.

Materials and methods

Organism and pre-cultivation

P. tricornutum Bohlin (CCMP2558, NCMA, Maine, USA) was obtained from the department Ocean Ecosystems of the Faculty of Science and Engineering of the University of Groningen. The culture was grown on a standard f/2-medium by the protocol of Guillard (Guillard 1975) with added $NaHCO_3$ to prevent carbon limitation. The end concentrations were 880µM N, 36µM P, 100 µM Si and 2.38mM $NaHCO_3$. Before every experiment a culture was pre-cultivated to the experimental conditions for at least 4-5 generations. The culture was first acclimated to the temperature conditions and then to the light conditions. In the pre-cultivation and at every experiment the light : dark cycle was 16:8h.

Experimental setup

Three sets of experiments were performed to determine the effects of irradiance and temperature on the fucoxanthin content, every experiment was done three times (n=3). The first experiment was to determine the effect of irradiance on the fucoxanthin content and the growth rate, this experiment was performed at ten different light intensities at 20°C and 25°C. The second experiment was performed to see if there is a correlation of the absorption (the optical density of the culture) with the dry weight and cell count. This experiment was performed at 20°C and 25°C, at 20°C two different light intensities were tested and at 25°C one light intensity was tested. The third experiment was to see how fast the fucoxanthin is induced when the culture is first grown at high light intensities and then switched to low light intensities. This experiment was performed at 20°C.

Experiment 1: Relationship irradiance, growth rate and fucoxanthin content
For the first experiment *P. tricornutum* was grown in small plastic 60mL cell culture flasks of Greiner Bio-One (ref 690 160). In every flask 5mL of culture was added to 55mL of f/2-medium. The flasks were placed in a photosynthetron with ten different compartments. These compartments were shielded with neutral density screens, resulting in ten different light intensities, see table 1. The photosynthetron was placed in a temperature controlled water bath (±1°C). The compartments were closed on top and on the sides and open on the bottom, hereby the light source (SBP, JOLLY 2/S 252-94-CR) came only from one side. The irradiance per compartment was measured with an irradiance meter with cosine corrected quantum sensor (LI-250, LI-COR). Every flask was stirred at least twice a day with the caps on. The experiment was repeated two times to have a total of three replicates. The experiments were performed at 20°C and 25°C.

At 20°C cultures were harvested for pigment analysis at the end of the exponential growth. The end of the exponential growth was specified when a flask reached an Optical Density (OD) of 0.8 at 550nm, the OD was not yet corrected for the width of the flask (raw data). The OD was measured with an Varian Cary 3E UV-visible spectrophotometer (see *Absorption measurements*). The cultures were transferred to a new flasks with fresh f/2-medium when an OD^{550nm} of 0.9 (uncorrected data) was reached. The cultures were diluted to an OD^{550nm} of 0.05 (raw data). When a flask didn't reach the critical OD values within two to three weeks, the flask would then be harvested and transferred. At 25°C cultures were harvested and transferred after one week, due to time limitation. For most of the flasks this corresponded with the end of the exponential growth. At 8 µmoll m^{-2} s^{-1}, 20°C there are only two data points available because the sample of the duplicate wasn't taken

Experiment 2: Correlation Absorption with dry weight and cell count
In the second experiment 3x20mL of pre-cultured *P. tricornutum* and 3x900mL of f/2-medium were put in three Erlenmeyer flasks for a triplicate. At 20°C the cultures were grown at 20 and 350 µmoll m^{-2} s^{-1}, at 25°C the cultures were grown at 150 µmoll m^{-2} s^{-1}, see table 1 for an overview. The experiment at low light conditions was put in a temperature controlled climate room (±1.5° C) under a single light source (4x Osram Biolux L 36W/965, with Doublelux reflectors) from above. The light intensity was measured with an irradiance meter with cosine corrected quantum sensor (LI-250, LI-COR). The experiments at high and medium light conditions were put in a temperature controlled u-shaped water bath (±1°C) where the light source (12x Osram Biolux L 36W/965, with Doublelux reflectors) came from the sides and the bottom. The light intensity was measured with a Quantum Scaler Irradiance Meter (QSL-100, Biospherical Instruments) just above the water level in the water bath. Everyday a sample of 55mL was put into a 60mL cell culture flask, Greiner Bio-One (ref 690 160), and the absorption, dry weight and cell count was measured (see *Dry weight measurement* and *Cell counts*). All the measurements were done before, during and after the exponential growth phase.

Experiment 3: Pigment induction
For the last experiment, the flasks grown at 20°C, 320 µmoll m^{-2} s^{-1} from the second experiment were used. Just after the exponential growth rate the light intensity was lowered to 20 µmoll m^{-2} s^{-1}, the temperature stayed the same. The algae were kept in this condition for fourteen days. Pigment samples were taken just before the light intensity was lowered and almost every day in the low light conditions. The absorption was measured every day. No new medium was added.

Experiment	*Temperature (°C)*	*Light (µmoll m^{-2} s^{-1})*	*Flask*	*mL algae – mL f/2*
1	20	8;17;27;36;65;82;134;169;317;516	Cell culture flask	5 – 55
1	25	5,5;14;22;32;58;69;108;134;259;480	Cell culture flask	5 – 55
2	20	~20	Erlenmeyer	20 – 900
2	25	~150	Erlenmeyer	20 – 900
2 / 3	20	~320 → ~20	Erlenmeyer	20 – 900

Table 1. *Different cultivation setups for every type of experiment. For experiment 2 and 3 the approximate light intensities are given, they varied ±2 µmoll m^{-2} s^{-1} depending on the position of the flask. The setup at 20°C and 350 µmoll m^{-2} s^{-1} was used for experiment 2 and 3.*

Absorption measurement

The entire cell culture flasks were put in a Varian Cary 3E UV-visible spectrophotometer and measured at wavelengths of 550nm, 680nm, 720nm and 750nm. The flasks had a width of two centimetres, the measured data was divided by 2 to obtain the OD per centimetre.

Dry weight determination

GF/C filters were dried beforehand in a stove at 95° C for an hour and a half and then weighed. The filters were placed on a vacuum pomp which created a pressure of -0,2 bar. Depending on the density of the culture, 40mL for thin culture or 25mL for a dense culture, the algae were filtered. To wash away the salt on the filters, which affects the dry weight, the filters were flushed with 0.5M NH_3HCO_3 according to Zhu & Lee (1997). The amount of NH_3HCO_3 filtered was half the amount of the filtered algae. The filters were then dried in a stove at 95° C for an hour and a half and then weighed again.

Cell counts

A 1-2mL sample was placed on a counting frame (Fuchs Rosenthal, 0,200mm x 0,0625mm^2) and a cover glass was put on top of it. The cells were allowed to settle down for at least half an hour before counting. Cells were counted on a counting frame under a normal light microscope. At least 300 cells were counted with a counter. Knowing the amount of cells, the amount of frames and the volume of a frame, the amount of cells per mL could be calculated.

Growth rate calculations

The growth rate was calculated from the absorption measurements, which were corrected for the width of the flasks. Average growth rates were calculated from the linear regression in the exponential phase of the natural logarithm of OD^{750nm} versus time. If a culture didn't reach critical OD values, the growth rate was calculated over the whole range. The growth rates where then modelled with an P:I curve based on (Frenette et al. 1993).

Pigment sampling and analysis

A 5mL sample was filtered through a GF/F filter (25mm, max pressure -0,2 bar). After filtering the sample was folded once and put in liquid nitrogen until completely frozen. The frozen filter with algae was then placed in a marked piece of aluminium foil and put back into the liquid nitrogen to prevent defrosting. When all the samples were taken they were stored in a -80° C freezer until analysing with the HPLC.

Before analysing, the filters were freeze dried and put into extraction fluid. The filters were freeze dried for 48 hours at -50° C and a pressure of $30*10^{-3}$ mbar. A small amount of liquid nitrogen was added to the containers with the filters to ensure the filters were kept frozen when starting up the freeze dryer. After freeze drying the filters were put into dark brown tubes, under dim light

conditions, with 5mL cold 90% acetone for 48 hours and stored at 4°C for extraction. After extraction, the fluid was analysed with the HPLC. The HPLC used was a Waters liquid chromatography (Model 2695), a cooled auto-sampler (4°C) and a Waters 996 diode-array detector. The freeze drying, extraction and HPLC analysing method is based on van Leeuwe et al. (2006). The regression of dry weight versus OD^{750} was used to calculate the specific dry weight at a certain absorption level. Knowing the dry weight, the fucoxanthin content could be calculated. Two regressions were used, one which contains all the data from the experiments at 20°C and the other contains all the data from the experiments at 20°C and 25°C. The first regression was used to calculate the fucoxanthin content for the experiments at 20°C and the second regressions was used to calculate the fucoxanthin content for the experiments at 25°C. On day 3 only two data points were available.

Statistics

IBM SPSS Statistics 24 was used for conducting all statistical analyses. Difference between treatments were analysed with an one-way analysis of variance (ANOVA) with an p-value of 0.05. Post hoc tests (Tukey HSD) were performed for pair-wise comparisons. For determining the relationship between dry weight with OD and cell count with OD a multiple linear regression was used with an p-value of 0.05.

Results

Effect of irradiance and temperature on growth rate

The different light intensities were split into different groups, Low Light (LL), Medium Light (ML) and High Light (HL), see *Appendix I* table 1 and 2. For both temperatures growth rates were significantly higher at ML and HL compared to LL ($p < 0.01$). Comparing ML with HL at both temperatures shows there is no significant difference in the growth rate ($p = 0.479$ at 20°C, $p = 0.586$ at 25°C). At high light intensities (ML/HL) growth rate was significantly higher at 25°C compared to 20°C ($p < 0.01$), at low light intensities (LL) there was no significant difference ($p = 0.890$).

Light is saturating at 100 µmoll m^{-2} s^{-1} for 20°C with an maximum growth rate of 0.95 day^{-1}, for 25°C light is saturating at 175 µmoll m^{-2} s^{-1} with an maximum growth rate of 1.41 day^{-1}.

Linear regression OD with cell counts and dry weight

The regression of OD with cell counts and OD with dry weight shows large variabilities in strength at the different wavelengths, but are all significant ($p < 0.01$) (see *Appendix II* table 5 and 6). At OD^{750nm} the regression with cell counts and dry weight is the strongest. Figure 2 shows the different regressions of cell count vs OD^{750nm} (A and B) and dry weight vs OD^{750nm} (C and D).

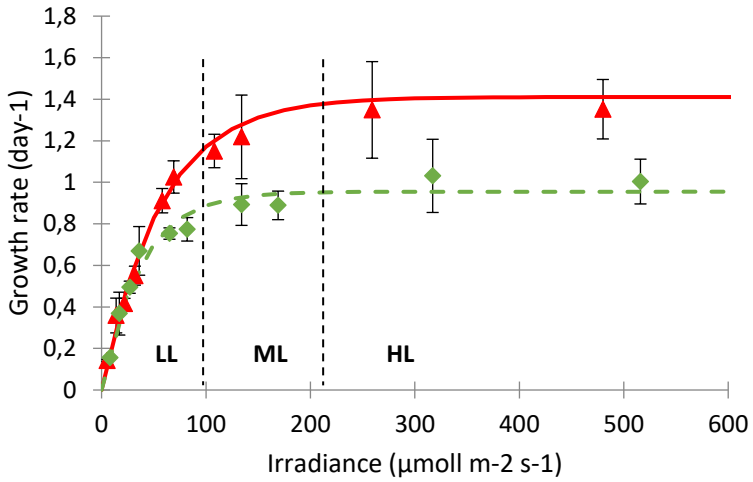

Figure 1. *Modelled growth rate vs irradiance curve of the mean growth rates with standard deviations at 25°C (triangles) and 20°C (diamonds). The vertical lines indicates the different groups.*

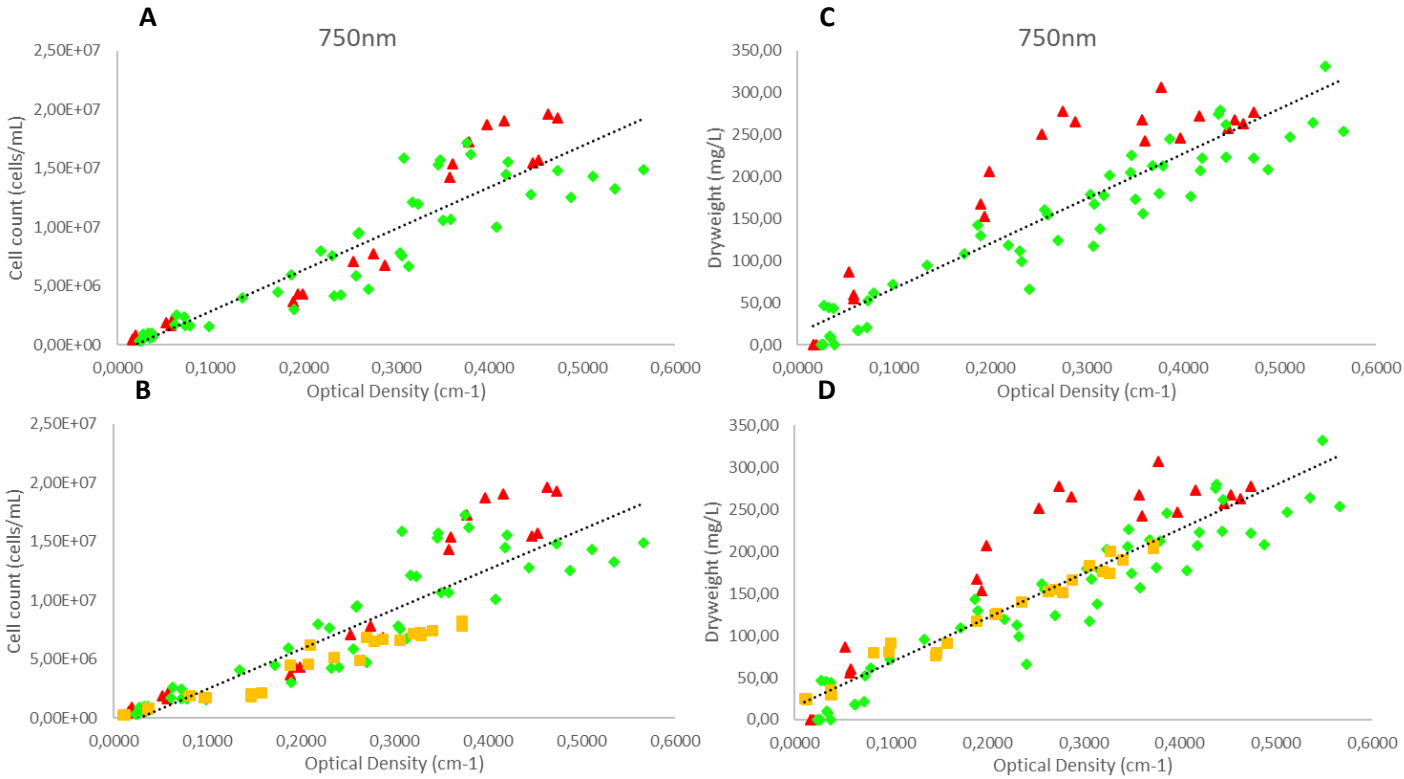

Figure 2. *Linear regressions for cell count vs OD^{750nm} (A and B) and dry weight vs OD^{750nm} (C and D). Triangles are for High Light data points (HL, 320 µmoll m^{-2} s^{-1}), diamonds for Low Light data points (LL, 20 µmoll m^{-2} s^{-1}) and squares for Medium Light data points (ML, 150 µmoll m^{-2} s^{-1}).* ***A.*** *shows the regression for cell counts vs OD^{750nm} for HL and LL (R^2 = 0.837, $p < 0.01$) at 20°C.* ***B.*** *shows the regression for cell counts vs OD^{750nm} for HL and LL at 20°C and ML at 25°C (R^2 = 0.817, $p < 0.01$).* ***C.*** *shows the regression for Dry weight vs OD^{750nm} for HL and LL (R^2 = 0.831, $p < 0.01$) at 20°C.* ***D.*** *shows the regression for dry weight vs OD^{750nm} for HL and LL at 20°C and ML at 25°C (R^2 = 0.855, $p < 0.01$).*

Effect of light and temperature on fucoxanthin content

The different light intensities were split into different groups, Low Light (LL), Medium Light (ML) and High Light (HL), see *Appendix III* table 8 and 9. Figure 3 shows a chart with the fucoxanthin content per biomass vs irradiance for 20°C and 25°C. At 20°C and 25°C irradiance had a significant effect on the fucoxanthin content. Fucoxanthin contents were significantly lower at ML and HL compared to LL ($p < 0.01$). Comparing HL with ML shows a significantly lower fucoxanthin content at HL for both temperatures ($p < 0.01$ at 20°C, $p = 0.017$ at 25°C). Temperature also has a significant effect on the fucoxanthin content, but only in high light conditions. At high light intensities (ML/HL) fucoxanthin content was significantly lower at 25°C ($p = 0.021$ for ML, $p < 0.01$ for HL), at low light intensities (LL) there was no significant difference ($p = 0.704$) between 20°C and 25°C.

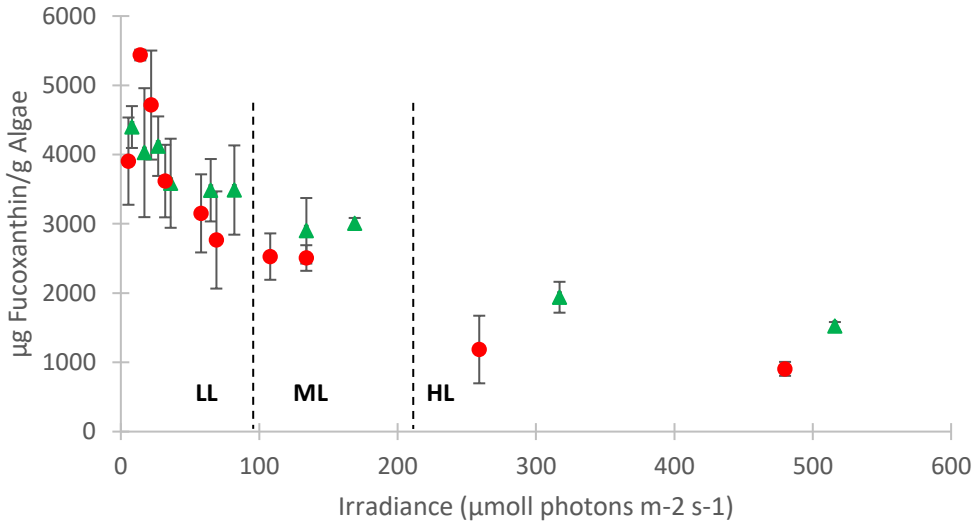

Figure 3. *Fucoxanthin content per biomass at different irradiances for 20°C (triangles) and 25°C (circles) with their respective standard deviations. The vertical lines indicate the different groups.*

Pigment induction

Figure 4 shows the fucoxanthin increase over time just before and fourteen days after lowering the irradiance. Day 1 is sampled right before the light conditions were lowered, day 2 is the first complete day in low light conditions. At day 6 the fucoxanthin content was significantly higher compared to day 1 ($p = 0.015$), from day 8 and onward the p-value is lower than 0.01 in comparison to day 1. There is no significant difference in fucoxanthin content from day 6 to day 15 ($p > 0.05$). The algal biomass didn't significantly change in the course of 15 days (figure 5).

Figure 6 shows the calculated relative increase in the fucoxanthin gain per day when placed in low light conditions. The increase is based on the mean values of the triplet at day 1. Day 1 is the last day in high light conditions just before it is placed in low light conditions, day 2 is the first complete day in low light conditions. The fucoxanthin gain is faster from day 1 to day 8 compared to day 8 to day 15.

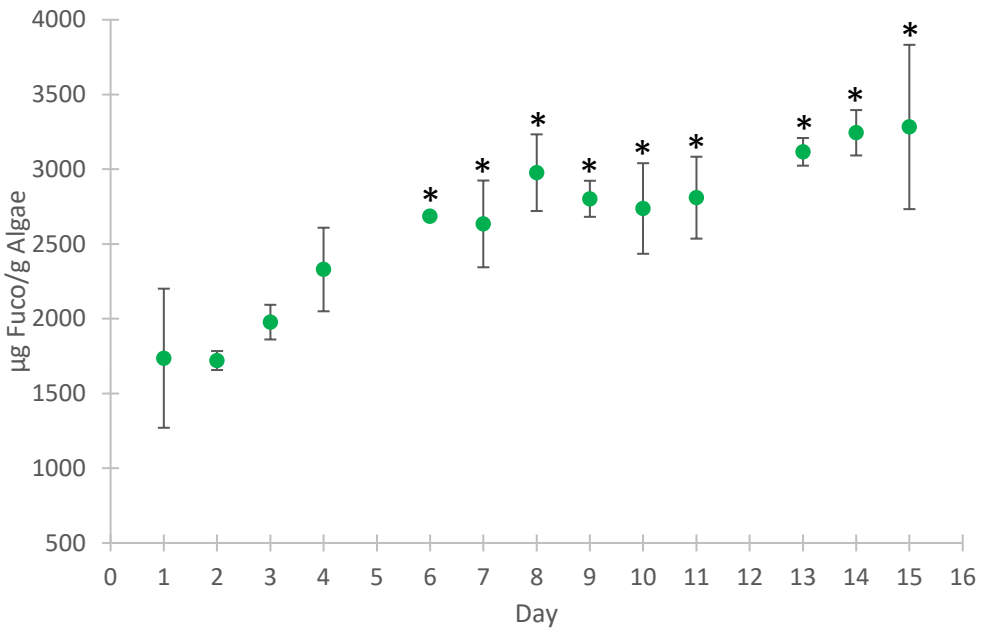

Figure 4. *Increase in fucoxanthin content per biomass over 15 days after the light was switched from high light conditions (320 µmoll m^{-2} s^{-1}) to low light conditions (20 µmoll m^{-2} s^{-1}) with their respective standard deviations. A star (*) indicates a significant higher fucoxanthin content per biomass on the specific day compared to day 1.*

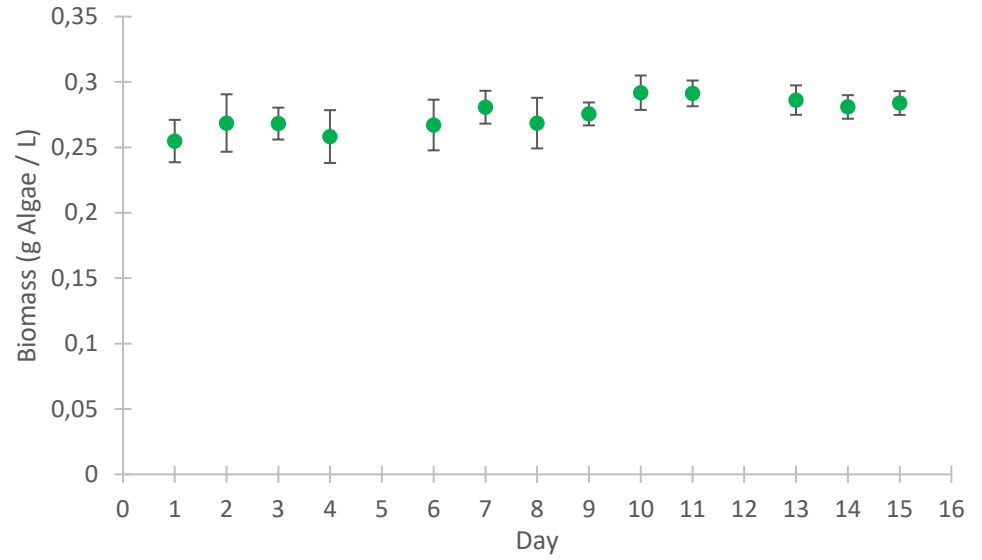

Figure 5. *The algal biomass (g Algae / L) over the course of 15 days with their respective standard deviations. There is no significantly increase or decrease in the algal biomass.*

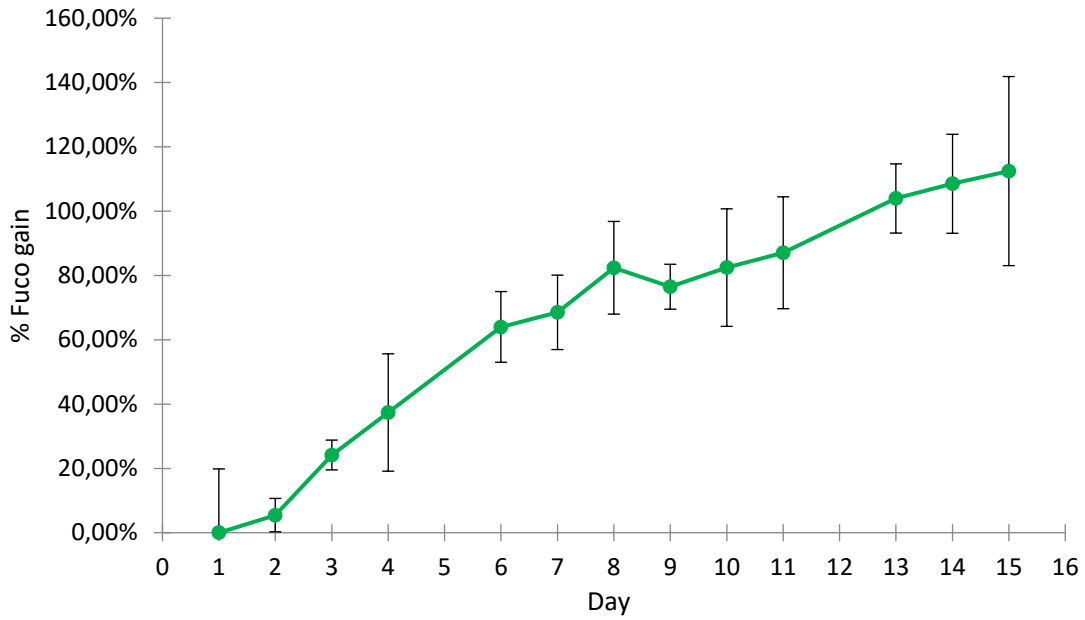

Figure 6. *Calculated fucoxanthin gain per day compared to day 1, with day 1 being the last day in high light conditions and day 2 the first complete day in low light conditions.*

Discussion

In this study the effect of irradiance and temperature on the fucoxanthin content in *P. tricornutum* and a potential optimal production process were studied. For this, several factors needed to be determined such as the growth rate, dry weight, cell counts and the fucoxanthin content. Higher irradiances showed a higher growth rate until a saturation point was reached, around 100 µmoll m^{-2} s^{-1} at 20°C and 175 µmoll m^{-2} s^{-1} at 25°C. Above these irradiances, irradiance was saturating and didn't have an effect on the growth rate. Also temperature had an effect on growth rates, at 25°C ML and HL had higher growth rates in comparison with 20°C, LL didn't differ between the two temperatures. These effects were in line with findings from Raven & Geider (1988) and Montagnes & Franklin (2001). Also the OD at 750nm showed to be a good linear predictor for the dry weight, which then could be used to calculate the fucoxanthin content per biomass.

Irradiance also has an effect on the amount of fucoxanthin. The fucoxanthin content decreased when the irradiance was increased, this was in line with the findings of MacIntyre & Geider (1996) and Laviale & Neveux (2011), but it only occurred at high light intensities (ML/HL). Higher temperature also had a negative effect on the fucoxanthin content in *P. tricornutum*, but only at ML and HL irradiances. As argued before, higher temperature may be an extra stressor or it may reduce the energy needed for the photosynthesis process, which could lead to lower light harvesting pigment content. These are new findings and no literature could be found on this subject. The combined effects of temperature and irradiance on the fucoxanthin content could have an impact in the (commercial) cultivation of *P. tricornutum*. Algae are often cultivated outside in the open where temperature and light intensity can vary greatly during the day and during the seasons. Especially when algae are grown in plastic bags the temperature inside may rise to unfavourable conditions, if not cooled. These results suggest to cultivate *P. tricornutum* outside the summer period to avoid temperatures inside the cultivation bags above 25°C and to apply a shader when the irradiance becomes too high.

The results from the first experiment were used in the third experiment for a possible optimal pigment production. *P. tricornutum* was first grown under high light intentsities which lowered the fucoxanthin content but increased the growth rate. At the end of its exponential growth phase, *P. tricornutum* was placed under low light conditions which increased it fucoxanthin content. At day 6 (after 5 days) the fucoxanthin content was significantly higher than day 1, but from day 6 and onward the fucoxanthin content didn't differ from day 6. This indicates a growth regime of growing *P. tricornutum* in high light conditions at 20°C for 4-5 days and subsequently 5 days in low light conditions. A calculation on the fucoxanthin gain per day, compared to day 1, based on these results shows an 64% increase in the fucoxanthin yield on day 6. At day 8 (7 days in low light) the model shows a little peak and predicts an increase of 82%, but according to the data of the third experiment this is not significantly higher compared to day 6. Only after 12 days in low light (day 13) the increase is over 100%.

A cultivation system with two compartments, growing algae in a plastic bag on top and storing it for fucoxanthin production beneath, should favour more fucoxanthin production. However the results suggest an increase of 64% in fucoxanthin yield per litre after 5 days in low light, whereas a second batch could be grown which accumulates to a 100% increase in fucoxanthin gain in the same time frame. Several adjustments can be made to potentially increase the fucoxanthin gain. According to Xia et al. (2013), growing the marine diatom *Odontella aurita* in a nitrogen-replete (18mM) L1-medium increase the fucoxanthin yield at 100 µmol m^{-2} s^{-1} and 300 µmol m^{-2} s^{-1} compared to a nitrogen-limited (6mM) L1-medium. These results should also be tested for *P. tricornutum*, but the extra nitrogen should be added to the second compartmen of the cultivation system to optimize the fucoxanthin production, because *P. tricornutum* already grows fast on the medium and the aim is only to increase the fucoxanthin content. However adding extra nitrogen reduces the sustainability. A second option could be to grow the algae at 25°C where the growth rate is higher but the fucoxanthin content in low light doesn't differ from 20°C. The total time needed for a complete cycle could then be reduced which may lead to a profitable cultivation setup.

Since the effects of temperature on the production of fucoxanthin in *P. tricornutum* are largely unkown, the effects should be studied thorougly before designing an optimal cultivation setup. Lower temperatures may have different effects on the fucoxanthin content of *P. tricornutum*. Second, different regimes with growing in high light and subsequently placing in low light at different temperatures should be studied. The third step should then be to studie different combinations of growing in a high light conditions at a certain temperature and subsequently placing in low light conditions at a different temperature. Alongside these steps, the effect of nitrogen-repletion can be studied to investigate if it's worthwile to develop a sustainable cultivation setup. However all the results of this study are obtained from laboratory experiments at only two different temperatures which may not be representatitve for outside culturing, but provide a good start for further research and experiments.

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Appendix I: Growth Rate

Table 1. *Mean growth rate at 20°C at different irradiances sorted in three groups, Low Light (LL), Medium Light (ML) and High Light (HL).*

Group	*Light (µmoll m^{-2} s^{-1})*	*Growth rate (day^{-1}) (±STD)*	*Temperature (° Celsius)*
LL	8	0.15 ± 0.01	20
LL	17	0.37 ± 0.10	20
LL	27	0.49 ± 0.03	20
LL	36	0.67 ± 0.12	20
LL	65	0.75 ± 0.03	20
LL	82	0.77 ± 0.06	20
ML	134	0.89 ± 0.10	20
ML	169	0.89 ± 0.07	20
HL	317	1.03 ± 0.18	20
HL	516	1.00 ± 0.11	20

Table 2. *Mean growth rate at 25°C at different irradiances sorted in three groups, Low Light (LL), Medium Light (ML) and High Light (HL).*

Group	*Light (µmoll m^{-2} s^{-1})*	*Growth rate (day^{-1}) (±STD)*	*Temperature (° Celsius)*
LL	5.5	0.14 ± 0.01	25
LL	14	0.36 ± 0.08	25
LL	22	0.42 ± 0.03	25
LL	32	0.55 ± 0.05	25
LL	58	0.91 ± 0.06	25
LL	69	1.03 ± 0.08	25
ML	108	1.15 ± 0.08	25
ML	134	1.22 ± 0.20	25
HL	259	1.35 ± 0.23	25
HL	480	1.35 ± 0.14	25

Table 3. *ANOVA-output of the one-way-ANOVA test which shows that at both temperatures the growth rate (day^{-1}) significantly differs between the different groups.*

u day-1

Temperature		Sum of Squares	df	Mean Square	F	Sig.
T20	Between Groups	1.148	2	0.574	16.506	0.000
	Within Groups	0.904	26	0.035		
	Total	2.052	28			
T25	Between Groups	3.915	2	1.957	23.613	0.000
	Within Groups	2.321	28	0.083		
	Total	6.236	30			

Table 4. *Post-Hoc (TUKEY HSD) output of the one-way-ANOVA test which groups at both temperatures significantly differ in growth rates (day^{-1}).*

Tukey HSD

Temperature			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
T20	LL	ML	-.33220*	0.08854	0.002	-0.5522	-0.1122
		HL	-.45860*	0.08854	0.000	-0.6786	-0.2386
	ML	LL	.33220*	0.08854	0.002	0.1122	0.5522
		HL	-0.12640	0.10765	0.479	-0.3939	0.1411
	HL	LL	.45860*	0.08854	0.000	0.2386	0.6786
		ML	0.12640	0.10765	0.479	-0.1411	0.3939
T25	LL	ML	-.63917*	0.13483	0.000	-0.9728	-0.3056
		HL	-.80462*	0.13483	0.000	-1.1382	-0.4710
	ML	LL	.63917*	0.13483	0.000	0.3056	0.9728
		HL	-0.16545	0.16623	0.586	-0.5768	0.2459
	HL	LL	.80462*	0.13483	0.000	0.4710	1.1382
		ML	0.16545	0.16623	0.586	-0.2459	0.5768

*. The mean difference is significant at the 0.05 level.

Table 5. *ANOVA-output of the one-way-ANOVA test which shows the difference in the growth rate (day^{-1}) per group between the different temperatures (20°C and 25°C).*

u day-1

Light		Sum of Squares	df	Mean Square	F	Sig.
LL	Between Groups	0.002	1	0.002	0.019	0.890
	Within Groups	2.737	34	0.081		
	Total	2.739	35			
ML	Between Groups	0.259	1	0.259	14.577	0.003
	Within Groups	0.178	10	0.018		
	Total	0.437	11			
HL	Between Groups	0.332	1	0.332	10.716	0.008
	Within Groups	0.310	10	0.031		
	Total	0.643	11			

Appendix II: Cell counts and dry weight

Table 6. *Linear regressions formula for cell counts (y) versus optical density (x) at every wavelength. The linear regressions formula are for the experiments at High Light (350 µmoll m^{-2} s^{-1}) with Low Light (20 µmoll m^{-2} s^{-1}) at 20°C and High Light (320 µmoll m^{-2} s^{-1}) with Low Light (20 µmoll m^{-2} s^{-1}) at 20°C and Medium Light (150 µmoll m^{-2} $s^{-}1$) at 25°C.*

Wavelength (nm)	*Formula cell counts (cells/mL)*	*R^2*	*p-value*	*Light (µmoll m^{-2} s^{-1})*	*Temperature (° Celsius)*
550	y = 2.53e+7x - 4.91e+5	0.815	<0.01	20 + 320	20
680	y = 2.40e+7x - 1.30e+5	0.784	<0.01	20 + 320	20
720	y = 3.28e+7x - 6.34e+5	0.830	<0.01	20 + 320	20
750	y = 3.52e+7x - 6.44e+5	0.837	<0.01	20 + 320	20
550	y = 2.42e+7x - 8.39e+5	0.783	<0.01	20 + 150 + 320	20 + 25
680	y = 2.34e+7x - 5.52e+5	0.776	<0.01	20 + 150 + 320	20 + 25
720	y = 3.16e+7x - 9.17e+5	0.809	<0.01	20 + 150 + 320	20 + 25
750	y = 3.39e+7x - 9.21e+5	0.817	<0.01	20 + 150 + 320	20 + 25

Table 7. *Linear regressions formula for dry weight (y) versus optical density (x) at every wavelength. The linear regressions formula are for the experiments at High Light (350 µmoll m^{-2} s^{-1}) with Low Light (20 µmoll m^{-2} s^{-1}) at 20°C and High Light (320 µmoll m^{-2} s^{-1}) with Low Light (20 µmoll m^{-2} s^{-1}) at 20°C and Medium Light (150 µmoll m^{-2} $s^{-}1$) at 25°C.*

Wavelength (nm)	*Formula dry weight (mg/L)*	*R^2*	*p-value*	*Light (µmoll m^{-2} s^{-1})*	*Temperature (° Celsius)*
550	y = 3.76e+2x + 18.41	0.794	<0.01	20 + 320	20
680	y = 3.51e+2x + 25.40	0.752	<0.01	20 + 320	20
720	y = 4.94e+2x + 15.14	0.820	<0.01	20 + 320	20
750	y = 5.33e+2x + 14.36	0.831	<0.01	20 + 320	20
550	y = 3.75e+2x +16.68	0.822	<0.01	20 + 150 + 320	20 + 25
680	y = 3.55e+2x + 23.72	0.789	<0.01	20 + 150 + 320	20 + 25
720	y = 4.91e+2x + 15.57	0.845	<0.01	20 + 150 + 320	20 + 25
750	y = 5.29e+2x + 15.17	0.855	<0.01	20 + 150 + 320	20 + 25

Appendix III: Effect of light and temperature on fucoxanthin content

Table 8. *Fucoxanthin content per biomass at 20°C at different irradiances sorted in three groups, Low Light (LL), Medium Light (ML) and High Light (HL).*

Group	*Light (µmoll m^{-2} s^{-1})*	*µg Fuco / g algae (±STD)*	*Temperature (° Celsius)*
LL	8	4.4e+3 ±3.0e+2	20
LL	17	4.0e+3 ±9.3e+2	20
LL	27	4.1e+3 ±4.3e+2	20
LL	36	3.6e+3 ±6.4e+2	20
LL	65	3.5e+3 ±4.5e+2	20
LL	82	3.5e+3 ±6.4e+2	20
ML	134	2.9e+3 ±4.7e+2	20
ML	169	3.0e+3 ±7.9e+1	20
HL	317	1.9e+3 ±2.2e+2	20
HL	516	1.5e+3 ±5.9e+1	20

Table 9. *Fucoxanthin content per biomass at 25°C at different irradiances sorted in three groups, Low Light (LL), Medium Light (ML) and High Light (HL).*

Group	*Light (µmoll m^{-2} s^{-1})*	*µg Fuco / g algae (±STD)*	*Temperature (° Celsius)*
LL	5.5	3.9e+3 ±6.3e+2	25
LL	14	5.4e+3 ±7.6e+1	25
LL	22	4.7e+3 ±7.9e+2	25
LL	32	3.6e+3 ±5.2e+2	25
LL	58	3.1e+3 ±5.6e+2	25
LL	69	2.8e+3 ±7.0e+2	25
ML	108	2.5e+3 ±3.3e+2	25
ML	134	2.5e+3 ±1.8e+2	25
HL	259	1.2e+3 ±4.8e+2	25
HL	480	0.9e+3 ±1.0e+2	25

Table 10. *ANOVA-output of the one-way-ANOVA test which shows that at both temperatures the fucoxanthin content (µg Fuco / g algae) significantly differs between the different groups.*

µg Fuco / g algae

Temperature		Sum of Squares	df	Mean Square	F	Sig.
T20	Between Groups	19811157.849	2	9905578.925	36.784	0.000
	Within Groups	7001580.722	26	269291.566		
	Total	26812738.571	28			
T25	Between Groups	39803429.922	2	19901714.961	26.925	0.000
	Within Groups	19957085.240	27	739151.305		
	Total	59760515.162	29			

Table 11. *Post-Hoc (TUKEY HSD) output of the one-way-ANOVA test which groups at both temperatures significantly differ in the fucoxanthin content (µg Fuco / g algae).*

Dependent Variable:	µg Fuco / g algae	Tukey HSD					
Temperature			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
T20	LL	ML	865.95442*	246.41958	0.005	253.6279	1478.2809
		HL	2087.18259*	246.41958	0.000	1474.8561	2699.5091
	ML	LL	-865.95442*	246.41958	0.005	-1478.2809	-253.6279
		HL	1221.22817*	299.60617	0.001	476.7387	1965.7177
	HL	LL	-2087.18259*	246.41958	0.000	-2699.5091	-1474.8561
		ML	-1221.22817*	299.60617	0.001	-1965.7177	-476.7387
T25	LL	ML	1415.36473*	405.28489	0.005	410.4942	2420.2353
		HL	2886.49345*	405.28489	0.000	1881.6229	3891.3640
	ML	LL	-1415.36473*	405.28489	0.005	-2420.2353	-410.4942
		HL	1471.12872*	496.37060	0.017	240.4187	2701.8388
	HL	LL	-2886.49345*	405.28489	0.000	-3891.3640	-1881.6229
		ML	-1471.12872*	496.37060	0.017	-2701.8388	-240.4187

*. The mean difference is significant at the 0.05 level.

Table 12. *ANOVA-output of the one-way-ANOVA test which shows the difference in the the fucoxanthin content (µg Fuco / g algae) per group between the different temperatures (20°C and 25°C).*

µg Fuco / g algae						
Light		Sum of Squares	df	Mean Square	F	Sig.
LL	Between Groups	112162.351	1	112162.351	0.147	0.704
	Within Groups	25208210.304	33	763885.161		
	Total	25320372.655	34			
ML	Between Groups	570667.411	1	570667.411	7.408	0.021
	Within Groups	770310.239	10	77031.024		
	Total	1340977.650	11			
HL	Between Groups	1411975.528	1	1411975.528	14.406	0.004
	Within Groups	980145.418	10	98014.542		
	Total	2392120.946	11			

Appendix IV: Pigment induction

Table 13. *Fucoxanthin content for every single day and the calculated gain per day and the percentage gain per day.*

Day	*Fucoxanthin content (µg pigment L^{-1})*	*Fucoxanthin gain per day (µg pigment L^{-1} day^{-1})*	*Fucoxanthin gain per day (% pigment gain L^{-1} day^{-1})*
1	271.27	0.00	0.00
2	292.56	21.30	7.85
3	335.41	64.15	23.65
4	372.02	100.75	37.14
6	452.67	181.40	66.87
7	479.82	208.56	76.88
8	506.06	234.79	86.55
9	496.03	224.76	82.86
10	529.02	257.75	95.02
11	541.50	270.23	99.62
13	584.60	313.33	115.51
14	591.52	320.25	118.06
15	608.43	337.16	124.29

Table 14. *ANOVA-output of the one-way-ANOVA test which shows there is a significant difference in the fucoxanthin content (µg Fuco / g algae) between at least two days in the pigment induction experiment.*

µg Fuco / g algae

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12892119.047	12	1074343.254	7.831	0.000
Within Groups	3566890.806	26	137188.108		
Total	16459009.853	38			

Table 15. *Post-Hoc (TUKEY HSD) output of the one-way-ANOVA test which shows the difference in fucoxanthin content (µg Fuco / g algae) between each day.*

Dependent Variable: µg Fuco / g algae — Tukey HSD

(I) Day		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	15.50000	302.42146	1.000	-1083.5109	1114.5109
	3.00	279.10333	302.42146	0.999	-819.9076	1378.1142
	4.00	-593.58000	302.42146	0.746	-1692.5909	505.4309
	6.00	-950.06667	302.42146	0.140	-2049.0776	148.9442
	7.00	-898.44000	302.42146	0.193	-1997.4509	200.5709

	8.00	-1241.11000*	302.42146	0.017	-2340.1209	-142.0991
	9.00	-1066.41333	302.42146	0.063	-2165.4242	32.5976
	10.00	-1001.63000	302.42146	0.099	-2100.6409	97.3809
	11.00	-1073.96667	302.42146	0.060	-2172.9776	25.0442
	13.00	-1380.63000*	302.42146	0.006	-2479.6409	-281.6191
	14.00	-1508.19000*	302.42146	0.002	-2607.2009	-409.1791
	15.00	-1546.85000*	302.42146	0.001	-2645.8609	-447.8391
2.00	1.00	-15.50000	302.42146	1.000	-1114.5109	1083.5109
	3.00	263.60333	302.42146	0.999	-835.4076	1362.6142
	4.00	-609.08000	302.42146	0.716	-1708.0909	489.9309
	6.00	-965.56667	302.42146	0.126	-2064.5776	133.4442
	7.00	-913.94000	302.42146	0.175	-2012.9509	185.0709
	8.00	-1256.61000*	302.42146	0.015	-2355.6209	-157.5991
	9.00	-1081.91333	302.42146	0.057	-2180.9242	17.0976
	10.00	-1017.13000	302.42146	0.089	-2116.1409	81.8809
	11.00	-1089.46667	302.42146	0.054	-2188.4776	9.5442
	13.00	-1396.13000*	302.42146	0.005	-2495.1409	-297.1191
	14.00	-1523.69000*	302.42146	0.002	-2622.7009	-424.6791
	15.00	-1562.35000*	302.42146	0.001	-2661.3609	-463.3391
3.00	1.00	-279.10333	302.42146	0.999	-1378.1142	819.9076
	2.00	-263.60333	302.42146	0.999	-1362.6142	835.4076
	4.00	-872.68333	302.42146	0.224	-1971.6942	226.3276
	6.00	-1229.17000*	302.42146	0.019	-2328.1809	-130.1591
	7.00	-1177.54333*	302.42146	0.028	-2276.5542	-78.5324
	8.00	-1520.21333*	302.42146	0.002	-2619.2242	-421.2024
	9.00	-1345.51667*	302.42146	0.007	-2444.5276	-246.5058
	10.00	-1280.73333*	302.42146	0.012	-2379.7442	-181.7224
	11.00	-1353.07000*	302.42146	0.007	-2452.0809	-254.0591
	13.00	-1659.73333*	302.42146	0.001	-2758.7442	-560.7224
	14.00	-1787.29333*	302.42146	0.000	-2886.3042	-688.2824
	15.00	-1825.95333*	302.42146	0.000	-2924.9642	-726.9424
4.00	1.00	593.58000	302.42146	0.746	-505.4309	1692.5909
	2.00	609.08000	302.42146	0.716	-489.9309	1708.0909
	3.00	872.68333	302.42146	0.224	-226.3276	1971.6942
	6.00	-356.48667	302.42146	0.991	-1455.4976	742.5242
	7.00	-304.86000	302.42146	0.998	-1403.8709	794.1509

	8.00	-647.53000	302.42146	0.637	-1746.5409	451.4809
	9.00	-472.83333	302.42146	0.926	-1571.8442	626.1776
	10.00	-408.05000	302.42146	0.973	-1507.0609	690.9609
	11.00	-480.38667	302.42146	0.918	-1579.3976	618.6242
	13.00	-787.05000	302.42146	0.357	-1886.0609	311.9609
	14.00	-914.61000	302.42146	0.175	-2013.6209	184.4009
	15.00	-953.27000	302.42146	0.137	-2052.2809	145.7409
6.00	1.00	950.06667	302.42146	0.140	-148.9442	2049.0776
	2.00	965.56667	302.42146	0.126	-133.4442	2064.5776
	3.00	1229.17000*	302.42146	0.019	130.1591	2328.1809
	4.00	356.48667	302.42146	0.991	-742.5242	1455.4976
	7.00	51.62667	302.42146	1.000	-1047.3842	1150.6376
	8.00	-291.04333	302.42146	0.998	-1390.0542	807.9676
	9.00	-116.34667	302.42146	1.000	-1215.3576	982.6642
	10.00	-51.56333	302.42146	1.000	-1150.5742	1047.4476
	11.00	-123.90000	302.42146	1.000	-1222.9109	975.1109
	13.00	-430.56333	302.42146	0.960	-1529.5742	668.4476
	14.00	-558.12333	302.42146	0.810	-1657.1342	540.8876
	15.00	-596.78333	302.42146	0.740	-1695.7942	502.2276
7.00	1.00	898.44000	302.42146	0.193	-200.5709	1997.4509
	2.00	913.94000	302.42146	0.175	-185.0709	2012.9509
	3.00	1177.54333*	302.42146	0.028	78.5324	2276.5542
	4.00	304.86000	302.42146	0.998	-794.1509	1403.8709
	6.00	-51.62667	302.42146	1.000	-1150.6376	1047.3842
	8.00	-342.67000	302.42146	0.993	-1441.6809	756.3409
	9.00	-167.97333	302.42146	1.000	-1266.9842	931.0376
	10.00	-103.19000	302.42146	1.000	-1202.2009	995.8209
	11.00	-175.52667	302.42146	1.000	-1274.5376	923.4842
	13.00	-482.19000	302.42146	0.916	-1581.2009	616.8209
	14.00	-609.75000	302.42146	0.714	-1708.7609	489.2609
	15.00	-648.41000	302.42146	0.635	-1747.4209	450.6009
8.00	1.00	1241.11000*	302.42146	0.017	142.0991	2340.1209
	2.00	1256.61000*	302.42146	0.015	157.5991	2355.6209
	3.00	1520.21333*	302.42146	0.002	421.2024	2619.2242
	4.00	647.53000	302.42146	0.637	-451.4809	1746.5409
	6.00	291.04333	302.42146	0.998	-807.9676	1390.0542
	7.00	342.67000	302.42146	0.993	-756.3409	1441.6809

	9.00	174.69667	302.42146	1.000	-924.3142	1273.7076
	10.00	239.48000	302.42146	1.000	-859.5309	1338.4909
	11.00	167.14333	302.42146	1.000	-931.8676	1266.1542
	13.00	-139.52000	302.42146	1.000	-1238.5309	959.4909
	14.00	-267.08000	302.42146	0.999	-1366.0909	831.9309
	15.00	-305.74000	302.42146	0.998	-1404.7509	793.2709
9.00	1.00	1066.41333	302.42146	0.063	-32.5976	2165.4242
	2.00	1081.91333	302.42146	0.057	-17.0976	2180.9242
	3.00	1345.51667*	302.42146	0.007	246.5058	2444.5276
	4.00	472.83333	302.42146	0.926	-626.1776	1571.8442
	6.00	116.34667	302.42146	1.000	-982.6642	1215.3576
	7.00	167.97333	302.42146	1.000	-931.0376	1266.9842
	8.00	-174.69667	302.42146	1.000	-1273.7076	924.3142
	10.00	64.78333	302.42146	1.000	-1034.2276	1163.7942
	11.00	-7.55333	302.42146	1.000	-1106.5642	1091.4576
	13.00	-314.21667	302.42146	0.997	-1413.2276	784.7942
	14.00	-441.77667	302.42146	0.953	-1540.7876	657.2342
	15.00	-480.43667	302.42146	0.918	-1579.4476	618.5742
10.00	1.00	1001.63000	302.42146	0.099	-97.3809	2100.6409
	2.00	1017.13000	302.42146	0.089	-81.8809	2116.1409
	3.00	1280.73333*	302.42146	0.012	181.7224	2379.7442
	4.00	408.05000	302.42146	0.973	-690.9609	1507.0609
	6.00	51.56333	302.42146	1.000	-1047.4476	1150.5742
	7.00	103.19000	302.42146	1.000	-995.8209	1202.2009
	8.00	-239.48000	302.42146	1.000	-1338.4909	859.5309
	9.00	-64.78333	302.42146	1.000	-1163.7942	1034.2276
	11.00	-72.33667	302.42146	1.000	-1171.3476	1026.6742
	13.00	-379.00000	302.42146	0.985	-1478.0109	720.0109
	14.00	-506.56000	302.42146	0.887	-1605.5709	592.4509
	15.00	-545.22000	302.42146	0.831	-1644.2309	553.7909
11.00	1.00	1073.96667	302.42146	0.060	-25.0442	2172.9776
	2.00	1089.46667	302.42146	0.054	-9.5442	2188.4776
	3.00	1353.07000*	302.42146	0.007	254.0591	2452.0809
	4.00	480.38667	302.42146	0.918	-618.6242	1579.3976
	6.00	123.90000	302.42146	1.000	-975.1109	1222.9109
	7.00	175.52667	302.42146	1.000	-923.4842	1274.5376
	8.00	-167.14333	302.42146	1.000	-1266.1542	931.8676

	9.00	7.55333	302.42146	1.000	-1091.4576	1106.5642
	10.00	72.33667	302.42146	1.000	-1026.6742	1171.3476
	13.00	-306.66333	302.42146	0.997	-1405.6742	792.3476
	14.00	-434.22333	302.42146	0.958	-1533.2342	664.7876
	15.00	-472.88333	302.42146	0.926	-1571.8942	626.1276
13.00	1.00	1380.63000*	302.42146	0.006	281.6191	2479.6409
	2.00	1396.13000*	302.42146	0.005	297.1191	2495.1409
	3.00	1659.73333*	302.42146	0.001	560.7224	2758.7442
	4.00	787.05000	302.42146	0.357	-311.9609	1886.0609
	6.00	430.56333	302.42146	0.960	-668.4476	1529.5742
	7.00	482.19000	302.42146	0.916	-616.8209	1581.2009
	8.00	139.52000	302.42146	1.000	-959.4909	1238.5309
	9.00	314.21667	302.42146	0.997	-784.7942	1413.2276
	10.00	379.00000	302.42146	0.985	-720.0109	1478.0109
	11.00	306.66333	302.42146	0.997	-792.3476	1405.6742
	14.00	-127.56000	302.42146	1.000	-1226.5709	971.4509
	15.00	-166.22000	302.42146	1.000	-1265.2309	932.7909
14.00	1.00	1508.19000*	302.42146	0.002	409.1791	2607.2009
	2.00	1523.69000*	302.42146	0.002	424.6791	2622.7009
	3.00	1787.29333*	302.42146	0.000	688.2824	2886.3042
	4.00	914.61000	302.42146	0.175	-184.4009	2013.6209
	6.00	558.12333	302.42146	0.810	-540.8876	1657.1342
	7.00	609.75000	302.42146	0.714	-489.2609	1708.7609
	8.00	267.08000	302.42146	0.999	-831.9309	1366.0909
	9.00	441.77667	302.42146	0.953	-657.2342	1540.7876
	10.00	506.56000	302.42146	0.887	-592.4509	1605.5709
	11.00	434.22333	302.42146	0.958	-664.7876	1533.2342
	13.00	127.56000	302.42146	1.000	-971.4509	1226.5709
	15.00	-38.66000	302.42146	1.000	-1137.6709	1060.3509
15.00	1.00	1546.85000*	302.42146	0.001	447.8391	2645.8609
	2.00	1562.35000*	302.42146	0.001	463.3391	2661.3609
	3.00	1825.95333*	302.42146	0.000	726.9424	2924.9642
	4.00	953.27000	302.42146	0.137	-145.7409	2052.2809
	6.00	596.78333	302.42146	0.740	-502.2276	1695.7942
	7.00	648.41000	302.42146	0.635	-450.6009	1747.4209
	8.00	305.74000	302.42146	0.998	-793.2709	1404.7509
	9.00	480.43667	302.42146	0.918	-618.5742	1579.4476

	10.00	545.22000	302.42146	0.831	-553.7909	1644.2309
	11.00	472.88333	302.42146	0.926	-626.1276	1571.8942
	13.00	166.22000	302.42146	1.000	-932.7909	1265.2309
	14.00	38.66000	302.42146	1.000	-1060.3509	1137.6709

*. The mean difference is significant at the 0.05 level.

Table 16. *The mean algal biomass (g algae / L) with their respective standard deviation for every single day.*

Day	***g Algae / L (+STD)***
1	2.55e-01 ± 1.62e-2
2	2.69e-01 ± 2.20e-2
3	2.68e-01 ± 1.22e-2
4	2.58e-01 ± 2.02e-2
6	2.67e-01 ± 1.94e-2
7	2.81e-01 ± 1.26e-2
8	2.69e-01 ± 1.94e-2
9	2.76e-01 ± 8.77e-3
10	2.92e-01 ± 1.32e-2
11	2.91e-01 ± 9.85e-3
13	2.86e-01 ± 1.13e-2
14	2.81e-01 ± 9.00e-3
15	2.84e-01 ± 9.09e-3

Table 17. *ANOVA-output of the one-way-ANOVA test which shows there is no significant difference in the algal biomass (g algae / L).*

g Algae / L

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.005	12	0.000	1.937	0.077
Within Groups	0.006	26	0.000		
Total	0.011	38			

Table 18. *Percentage fucoxanthin gain when placed in low light conditions, with day 1 the last day of high light and day 2 the first complete day in low light conditions.*

Day	***% Fucoxanthin gain (+ % STD)***
1	0.00 ± 19.84
2	5.47 ± 5.19
3	24.17 ± 4.65
4	37.39 ± 18.24
6	64.00 ± 10.99
7	68.53 ± 11.58
8	82.39 ± 14.40
9	76.50 ± 6.99
10	82.44 ± 18.27
11	87.04 ± 17.40
13	103.94 ± 10.75
14	108.52 ± 15.39
15	112.45 ± 29.40