Horticultural Production Chains Group

HPC-80439 Thesis Horticulture

LEDs grow; Pulsed lighting with LEDs

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Reg nr. 820916553080

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WAGENINGEN UNIVERSITY
PLANT SCIENCES

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A feasibillity study of using pulsed red LEDs as a source of assimilation light. Using *Lemna minor* as a model

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CONTENTS

PREFACE

I started with this project thinking it would be a continuation in the same line of work of that of Ad Lavrijsen, partly it turned out that way, working with LEDs for example, but for most of the project this is not the case. The subject for this research quickly changed when Ad started working on a research project for Philips at the horticultural production chains group. Part of this project consisted of finding out the effects of pulsed light on plant growth, this was something that interested me very much and because it does not really make sense to do two almost similar subjects at the same research group, I started working on this subject. Throughout this thesis I have had much support from the previous work of Ad and of his help, and I would like to thank him for that.

My supervisor on this project was Dr. Jeremy Harbinson; I would like to take the opportunity here to thank him. Because without his extensive knowledge on building electronic and other related equipment, this project would not have been possible. Furthermore the knowledge he has on chlorophyll fluorescence, radiation uptake of plants and photosynthesis were of prime importance for this project.

But most of all I want to thank my friend, Lonneke Lauwerijssen for her endless support of me and for the time she put in checking the spelling and grammar of this report, certainly when I hit a rough spot she was always there to lift my spirit.

For me this has been a very good chance to see how research is being done in the field of plant sciences, and in particular in horticulture. Furthermore I always had an interest in assimilation lighting in horticulture; this project has deepened my understanding of this subject and has taught me a few new things. The aspect of building my own equipment was also very nice, since I always like doing things with my hands.

Last but certainly not least I hope that you will enjoy reading about everything discussed in this report. But let me ensure you that this report by no means tries to give a complete and definite overview of everything there is to know on pulsed lighting. It is just a starting point for further research, and an interesting subject to read about.

Elias Meerwaldt
September 2004

SUMMARY

In the last few years the use of assimilation lighting in the Netherlands increased enormously, however the lights currently used for assimilation lighting are not the most efficient lights for plant growth. Therefore research is done into other light sources to be used for the growth of plants. One of these light sources on which investigations are centred, are light emitting diodes (LEDs). For this research LEDs where used to create a pulsed light system, which could provide pulsed light for plant growth. The proposed effects of pulsed light lie in the field of photosynthesis, and than especially between the light and the dark reaction. However, from literature the hypothesis was made that photosynthesis and thus the growth of plants under pulsed light can only approach, but never exceed photosynthesis and thus growth under continuous light with the same daily light integral under identical environmental conditions. To test this hypothesis an experiment was set-up where *Lemna* plants where grown in the same environment, 21 °C, 60% RH and a photoperiod of 16 hours, with the same daily light integral, 11.52 mol m^{-2} day^{-1}, but different light sources providing this daily light integral. One treatment consisted of fluorescent tubes, which provided a light intensity of 200 µmol m^{-2} s^{-1}, another treatment that consisted of 24 LEDs spread out over 300 cm^2 that provided light over an area of 500 cm^2, this treatment also had a light intensity of 200 µmol m^{-2} s^{-1}. The last treatment provided light in pulses and consisted of 48 LEDs spread out over 300 cm^2 to light an area of 500 cm^2, where the ‘on’-signal was 400 µmol m^{-2} s^{-1} and the ‘off’-signal was 0 µmol m^{-2} s^{-1}. With these light sources six experiments were set-up, where the frequency of the pulsed light ranged from 0.012 Hz till 120 Hz, with a difference of a factor ten between the experiments. These frequencies also were used to name the experiments in some graphs. The results of these experiments where not as expected, in that different effects occurred over the range of frequencies. At 1.28 Hz the area growth rate of the plants was higher than that of the control treatment, and there also was a higher leaf area ratio (LAR). However, at 0.012 and 0.12 Hz a higher relative area growth rate is combined with a lower LAR. At 12 and 120 Hz the relative area growth rate is equal to the control treatment, at 12 Hz the LAR is also equal, but at 120 Hz the LAR is much lower. The final conclusion reached from these data is that it is possible to grow plants under pulsed red LEDs, however some morphological features of the plants change, furthermore most of the time it is not beneficial to grow plants under the pulsed light, certainly not below a frequency of 1 Hz. A lot of interesting processes occur in the plants when they are grown under pulsed light that are not clearly understood yet, so this remains an area of considerable interest for future research. Furthermore microsecond pulses and a different duty cycle can also be of interest for future research.

1. INTRODUCTION

1.1 Assimilation lighting

The use of lamps for improvement of CO_2-assimilation (assimilation lighting) of greenhouse crops has increased enormously during the last 15 years in the Netherlands. The main reasons for the use of assimilation lighting, be it in the pot plant, cut flower or vegetable sector, are to ensure high product quality and high production, an even more important reason however is to ensure year-round production and quality level which meets the market demand (Marcelis et al., 2002). The LEI has calculated that in the year 1999 13% of the complete Dutch greenhouse area used assimilation lighting and that this had increased with one percent point a year since 1994 (Bakker et al., 2000). By 2002 they have calculated that 22% of the Dutch greenhouse area made use of assimilation lighting, and that this figure had increased with 1.7 percent point a year since 1994 (Knijff and Benninga, 2003). In a few years time the increase of assimilation lighting is enormous. This increase in area was mainly elicited by the increase of the pot plant and cut flower greenhouses which use assimilation lighting. Furthermore, the developments in the vegetable sector are going fast, but this area is still relatively small (Knijff and Benninga, 2003). In 2003 nearly 20 hectares of tomatoes were being produced under assimilation lighting, which is approximately one and a half percent of the total area of tomatoes (Meerwaldt, 2003). The prospect for the upcoming year, 2004, is that the area will increase to 60 or 65 hectares (Burg, 2003).

Besides this increase in area under assimilation lighting, the intensity and the lighting hours have also increased rapidly in the last years. The average intensity has reached 39 W m^{-2} and the average lighting hours per year have reached 3450 (Knijff and Benninga, 2003). Around 1990 these values where respectively 28 W m^{-2} and 2900-3000 hours per year (Bakker et al., 2000)

These developments in the Netherlands can also be noticed in other countries on the Northern Hemisphere, some are even ahead of the Netherlands. For example in Iceland tests with assimilation lighting have started already in 1975 and the vegetable growers there are presently using an intensity of 200 W m^{-2} (Jakupaj-de Snoo, 2004). In the province Quebec almost all lettuce growers and approximately 10% of cucumber and 15% of the tomato growers use assimilation lighting in wintertime (Lavrijsen, 2003; Dorais and Gosselin, 2002). In Denmark by 1992 assimilation lighting was used on 35 % of total greenhouse area and on 50 % of the ornamental area (Hendriks, 1992).

The most commonly used assimilation lights today are high pressure sodium lights (HPS). These lights were primarily developed for the illumination of roads and other public environments. And even therein they were not that efficient, approximately 20% of the incoming radiation is converted into visible radiation, everything else is dissipated as heat. Moreover, plants have a very different absorption spectrum from the human eye (Figure 1.1), which results in an even smaller efficiency for plant growth. The newest HPS lights have an energy conversion efficiency for plant growth of 38% (Pot, 2004). However, these lights where especially developed for the horticultural sector. This means that there is still a lot of energy which is converted into heat. This could be seen as being slightly advantageous, especially in the wintertime, since the greenhouse has to be heated then anyway (Brault et al., 1989; Lavrijsen, 2003). Due to the constant intensification of the lighting this becomes less useful. This means that the total energy efficiency of the greenhouse decreases, naturally this is not an ideal situation.

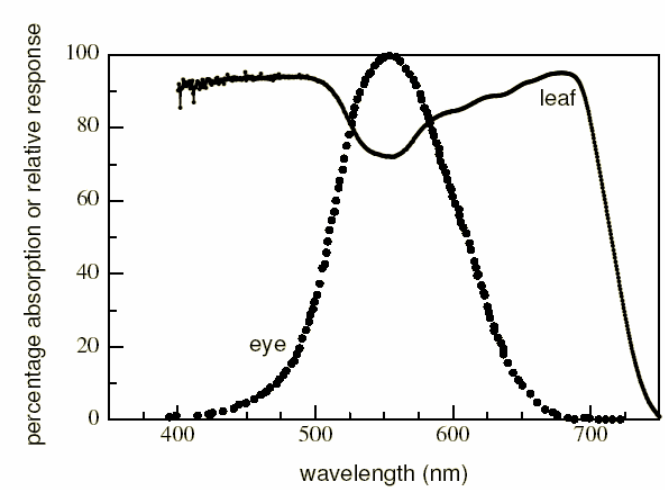

Figure 1.1. The standard CIE photopic response of a normal eye (eye) compared to the absorbance spectrum of a leaf (leaf). Especially interesting is the drop in absorbance of the leaf at around 550 nm which is why we see leaves as being green. (Harbinson and Rosenqvist, 2003)

Therefore research is done into new lighting possibilities for the horticultural sector. For example on microwave powered lamps (Kozai et al., 1995) and on light emitting diodes (LEDs) (Kim et al., 2004; Yorio et al., 2001; Yanagi and Okamoto, 1997; Lavrijsen, 2003). This research will focus on the latter, the LEDs. These LEDs are familiar to nearly everyone, because they are used as indicator lights of computers, keyboards, monitors, etc. Less known but also of importance is that they are used in backlighting of mobile phones and related devices.

LEDs are solid-state devices, which emit radiation in a very narrow wavelength range (a few nm), so called monochromatic light, which our eyes perceive as light from one colour. The low light output of LEDs and a lack of colour options have limited LED technology, to the uses mentioned earlier, in the past. Recently, new LED materials and improved production processes have resulted in bright LEDs in colours throughout the visible spectrum with efficacies greater than incandescent lamps (Craford, 2000). At present, typical indicator-LEDs have light outputs on the order of one to several lumens, whereas LEDs for illumination produce on the order of tens

to hundreds of lumens (Narendran et al., 2003). These brighter, more efficient and colourful LEDs are moving LED technology into a range of lighting applications (Bierman, 1998; Craford et al., 2001; Narendran et al., 2003). For example in traffic signals, car taillights, car turn signals, in interior design and large area displays (Craford, 2000; Craford et al., 2001).
These current applications and the promising future for LEDs as a replacement for light bulbs in many more applications (Craford et al., 2001; Narendran et al., 2003), and a fierce competition to produce the best LEDs, encourages engineers to continuously try to lower the cost of manufacturing LEDs and improve their light output and efficiency (Craford et al., 2001). The reported results for red LEDs are approximately of a 10-fold decrease in costs per lumen ($/lm) per decade and an approximately 30-fold increase in light output (lm) per decade (Craford, 2000). The efficiency of red LEDs increased from 10 lumens/Watt in 1990 to approximately 50 lumens/Watt in 2001 (Craford et al., 2001).

The above defined efficiency increase for LEDs is focused on the human perception of light, but the efficiency for plant growth is different as already has been shown in figure 1.1. This efficiency is given in the unit µmol s^{-1} $Watt^{-1}$. When looking at this efficiency the LEDs are already approaching HPS lamps. To be precise, the present efficiency of HPS lamps for plant growth is about 1.9 µmol s^{-1} $Watt^{-1}$, whereas the result of the latest calculations for LEDs is around 1.7 µmol s^{-1} $Watt^{-1}$ (Lavrijsen, 2004). Other calculations based on the existing LED arrays, which have been used in this research, showed an efficiency of 1.2 µmol s^{-1} $Watt^{-1}$ (1100 µmol on 0.05 m^2 = 55 µmol m^{-2} s^{-1} for 48 LEDs, so for 1 LED (= 1 Watt) = 55/48 = 1,15 µmol m^{-2} s^{-1}). So the LEDs are approaching the efficiency of HPS, and in a few years time it is envisioned that they will exceed the efficiency of HPS.

1.2 Photosynthesis

It is common knowledge that higher plants and other photosynthetic organisms, like cyanobacteria and algae, use light, carbon dioxide and water to create their own metabolic products. This is used, either direct or indirect, for our energy needs. The best known form of photosynthesis and also the one that will be described here, is the one carried out by higher plants and algae (equation (eq) 1.1).

$$nCO_2 + nH_2O \rightarrow (CH_2O)_n + nO_2 \qquad \text{(eq 1.1)}$$

This process can be divided in two reactions; the light reaction, where the light is captured, H_2O is transferred in H^+ and O^{2-} and energy is fixed in ATP and NADPH, and the dark reactions that uses the energy released from ATP and NADPH to convert CO_2 to $(CH_2O)_n$ (Lawlor, 1993a).

The light reaction and photon transport

Not all of the electromagnetic radiation emitted by the sun can be used in the light reaction, only a small part of the spectrum, called photosynthetically active radiation (PAR), can be used in the light reaction (figure 1.2).

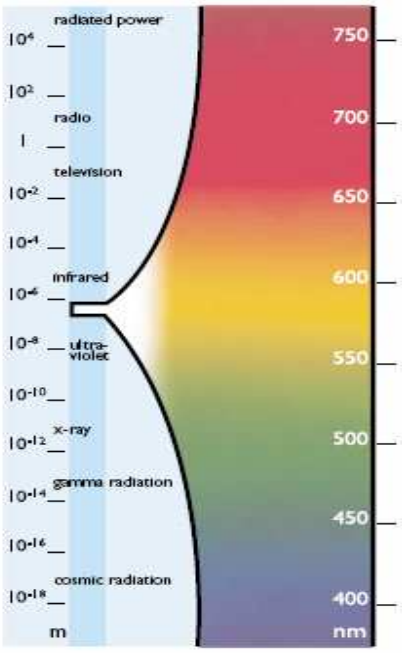

Figure 1.2. The electromagnetic radiation spectrum, the photosynthetically active radiation is enlarged (Pot and Leest, 2002).

The light reaction of photosynthesis occurs in a series of protein complexes bound to the thylakoid membrane of the chloroplast, which are interconnected by mobile electron carriers (Rosenqvist and Kooten, 2003). These protein complexes are the cytochrome b_6/f complex, the ATP synthase/hydrolase complex, photosystem II (PSII) and photosystem I (PSI). (Taiz and Zeiger, 2002; Rosenqvist and Kooten, 2003). The way the photosystems are described above is not the most obvious one. The reason for this is that photosystem I was the first to be discovered and then photosystem II secondly, however in the pathway photosystem II comes first.

Each photosystem consists of a huge area of light harvesting centres (LHCs) and a reaction centre. The energy entities in the LHC are called excitons. They are

capable of energizing the photosynthetic reaction centre chlorophyll dimer called P_{680} in PSII or P_{700} in PSI, where 680 and 700 denote the peak wavelength of absorption in the respective reaction centres (Rosenqvist and Kooten, 2003). The LHCs contain several types of pigments that absorb light of different parts of the electromagnetic radiation spectrum (photons with different energy content); although all light that is absorbed lies in the photosynthetically active radiation. Chlorophyll a and b absorb predominantly blue (≈ 400-450 nm) and red (≈ 650-700 nm) light (figure 1.3), whereas β-carotene (carotene) and lutien (xanthophyll) both caretenoids, absorb also light from the green part (≈ 450-550 nm) of the spectrum (Lavrijsen, 2003). In addition chlorophyll a absorbs in the blue part of the spectrum at lower wavelengths and in the red part of the spectrum at higher wavelengths than chlorophyll b (figure 1.3).

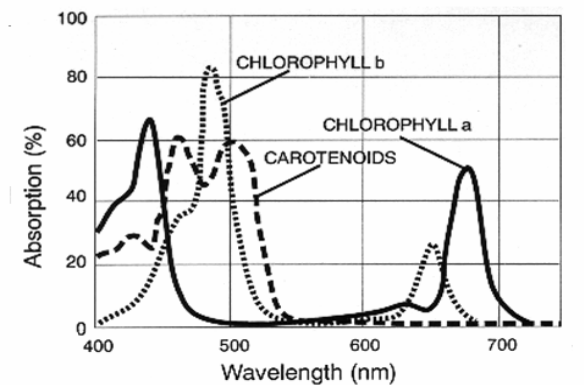

Figure 1.3. Estimated absorption spectra of chlorophyll a, chlorophyll b and carotenoids, the most important components of the LHCs (modified from Whitmarsh and Govindjee, 1999).

LHCI is linked to PSI and LHCII to PSII, these LHCs each consist of different combinations of the above described components, chlorophylls, carotenoids etc., this is why they have different absorption spectra. And this again determines why the photosystems each have a different absorption spectrum. In higher plants and green algae PSII absorbs more light than does PSI at wavelengths below about 670 nm till approximately 450 nm. At wavelengths longer than 670 nm and shorter than 450 nm, PSI absorption becomes predominant (figure 1.4). For maximum efficiency of photosynthesis, however it is important that both photosystems operate at approximately the same rate. On the short term this can be achieved by state transitions, they provide a mechanism whereby more balanced excitation of the two photosystems can be achieved (Fork and Satoh, 1986). On the longer term however, the photosystems react by changing the amount of PSI versus PSII (Rosenqvist and Kooten, 2003).

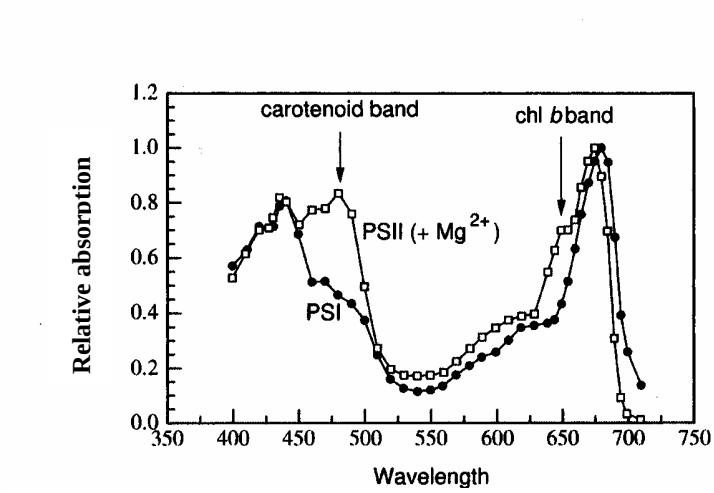

Figure 1.4. Comparative absorbances of photosystems I and II. The red absorbance peaks are 700 nm for PSI and 680 nm for PSII. The long wavelength tail of the PSI spectrum allows PSI to be preferentially excited by far-red light. (Modified from Harbinson and Rosenqvist, 2003)

It was once assumed that all of the light captured by the plants was used for photosynthesis, it has become clear however that this is not the case. When a photon is captured by an atom of the light harvesting complex, it becomes excited. Different colours of light have different wavelengths, and thus different energy levels, leading to different excitation levels; these have to be de-excited in their own special way (Figure 1.5).

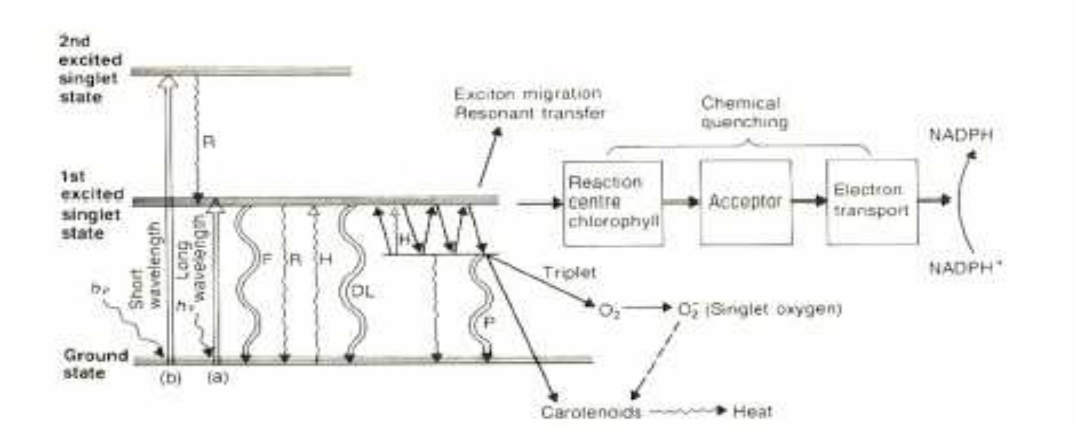

Figure 1.5. Concept of absorption of photons (h) by an atom, energizing an electron to an excited state (a) and its subsequent decay with release of energy. Capture of a more energetic photon (b) results in higher energy level orbitals being filled and then decay by radiationless transition (R). Heat (H) may also raise an electron to higher energy level and the energy is emitted when the electron drops back to the ground state. The main energy –dissipating processes are by radiationless transition (R), prompt fluorescence (F), delayed light emission (DL), phosphorescence (P) and by chemical reactions, for example, which are, in photosynthetic organisms assimilation of CO2 and transfer, for example of triplet energy to oxygen or caretonoids or of excitation energy to other chlorophyll and pigment molecules. (Lawlor, 1993b; Lavrijsen, 2003)

As shown in figure 1.5 the rate of decay of the excited state depends on radiationless transitions, fluorescence and photochemistry. These processes have rate constants for de-excitation, respectively, K_d, K_f and K_p so that the overall rate constant is:

$K= (K_d + K_f + K_p)$ (eq 1.2)

When n_0 excited states are present initially, the decrease to n excited states in time, t, is given by

$n = n_0 * e^{-Kt}$ (eq 1.3)

e is the base of the natural logarithm (Lawlor, 1993b).

The excitation of P_{680} and P_{700} causes ejection of chlorophylls from the RCs (oxidation) to primary electron acceptors, a chlorophyll a monomer for PSI (Schubert, 1997) and a pheophytin molecule for PSII. After that electron transport starts along the chain of redox components (Lawlor, 1993c). This electron transport results in water splitting, production of NADPH and acidification of the thylakoid lumen which drives the ATP synthesis by the ATP synthase/hydralase complex (Lawlor, 1993c). This non-cyclic electron transport and coupled processes is depicted in figure 1.6, and will not be further explained here, as it has been described well before (Lawlor, 1993c; Taiz and Zeiger, 2002).

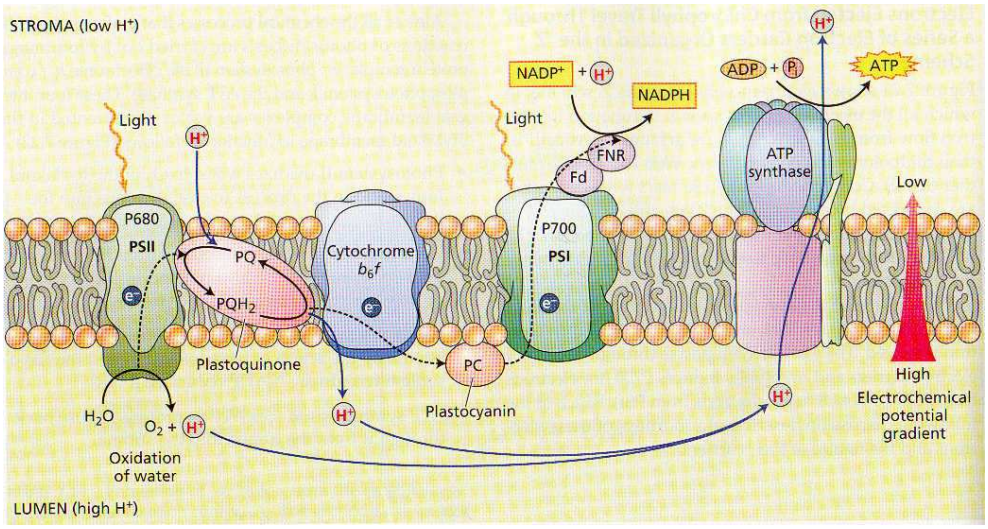

Figure 1.6. The transfer of electrons and protons in the thylakoid membrane is carried out vertically by four protein complexes. Water is oxidized and protons are released in the lumen by PSII. PSI reduces NADP+ to NADPH in the stroma, via the action of ferredoxin (Fd) and the flavoprotein ferredoxin-NADP reductase (FNR). Protons are also transported into the lumen by the action of the cytochrome b_6f complex and contribute to the electrochemical proton gradient. These protons must then diffuse to the ATP synthase enzyme, where their diffusion down the electrochemical potential gradient is used to synthesize ATP in the stroma. Reduced plastoquinone (PQH_2) and plastocyanin transfer electrons to cytochrome b_6f and to PSI, respectively. Dashed lines represent electron transfer; solid lines represent proton movement (Taiz and Zeiger, 2002; Lavrijsen, 2003).

The dark reaction

The dark reaction, although it is called in such a way does not need the dark to function, as is the case with the light reaction which needs the light, but it functions both in the light and in the dark, so a better name would be the light independent reaction. In the dark reaction the products produced in the light reaction (ATP and NADPH) provide the energy and electrons to reduce carbon dioxide to organic molecules. This process takes place in the so called Calvin cycle, the reaction takes place in the stroma of the chloroplast (eq 1.4).

$3\ CO_2 + 9\ ATP + 6\ NADPH \rightarrow$ glyceraldehyde-3-phosphate $+ 9\ ADP + 8\ Pi + 6\ NADP^+$ (eq 1.4)

Glyceraldehyde-3-phosphate may be converted to other carbohydrate metabolites (e.g., fructose-6-phosphate and glucose-1-phosphate), energy stores (e.g., sucrose or starch), or cell wall constituents (e.g., cellulose). Glyceraldehyde-3-P can also be utilized by plant cells as carbon source for synthesis of fatty acids and amino acids (Diwan, 2003).

The dark reactions are principally a series of chemical reactions and are much slower than the light reaction (picosecondscale (10^{-12} s)), therefore it might be beneficial to have a short dark period in between the light periods to make sure that the products made in the light reaction are all processed before new products arrive. But this will be discussed in more detail in the next paragraph (1.3).

Chlorophyll fluorescence

As already shown in figure 1.5, an absorbed photon can undergo multiple fates, in the leaf these are reduced to three main fates. Photons can be used for the photosynthesis, they can be dissipated as heat and they can be re-emitted as fluorescence. By measuring the yield of fluorescence one is able to say something about changes in deficiency of photochemistry and heat dissipation. Although only 1-2% of the absorbed photons are converted into fluorescence it is quite easy to measure (Maxwell and Johnson, 2000). Changes in fluorescence where first observed by Kautsky and co-workers in the early 1960's (Kautsky *et al.*, 1960). They discovered that if a leaf is transferred from the dark to the light an increase in fluorescence occurs in a time period of one second (Maxwell and Johnson, 2000). The rise in fluorescence can be explained by a reduction in electron acceptance in the photosynthetic pathway of photosystem II (PSII), where mainly the plastoquinone Q_A is reduced. When PSII absorbs light and Q_A accepts an electron, no other electrons can be taken up until Q_A passes its electron to Q_B. During this period the reaction

centre of PSII is closed. When a leaf is transferred from the dark to the light the Q_A pool is completely reduced and fluorescence is at its maximum (F_m) because the rate of electron transport (photochemical quenching) is constant and non photochemical quenching (NPQ) has not taken place yet. After then the fluorescence level decreases as a result of photochemical quenching and by NPQ (Schaftenaar, 2004).

Photochemical quenching parameters always relate to the relative value of F'_m and variable fluorescence (F_v). The most useful is the parameter that measures the efficiency of PSII photochemistry, ΦPSII (Maxwell and Johnson, 2000). This is calculated as (Rosenqvist and Kooten, 2003):

$$\Phi PSII = \Delta F' / F_m' = (F_m' - F_s') / F_m' \quad \text{(eq 1.5)}$$

With:

F' = fluorescence

F_m' = maximal fluorescence

F_s' = steady state fluorescence

This parameter measures the proportion of the light absorbed by the chlorophyll associated with PSII that is used in photochemistry. As such, it can give a measure of linear electron transport and so an indication of overall photosynthesis (Maxwell and Johnson, 2000).

Mostly fluorescence of PSII is measured because PSII has a higher fluorescence than photosystem I (PSI) and the yield of the fluorescence changes with changing physiological conditions. The fluorescence of PSI remains mostly quite stable (Schaftenaar, 2004).

1.3 Pulsed light

The first experiments on the application of light from electric lamps to plant growth marked the beginnings of an effort to improve the electrical efficiency and spectral properties of lamps for optimum plant growth and development. In these efforts use of intermittent light has been of major interest (Sager and Giger, 1980). The first ones to start with these experiments where Brown and Escombe (1905), who used a rotating segmented wheel to reduce irradiance by 25% without altering the spectral quality and rate of photosynthesis. In later articles use of intermittent radiation was proposed to increase yields, fasten growth rates, or both (Emerson and Arnold, 1932; Rabinowitch, 1956; Warburg, 1919). There are two factors which are important to describe the effect of intermittent light. These are the frequency of the pulses and the duty ratio of the intermittent light. The duty ratio is defined as the ratio between the duration of light-on (T_H) to the light-off (T_L) period (figure 1.7). Warburg (1919) found that the oxygen yield of *Chlorella* per amount of light, which is a measure for the efficiency of photosynthesis, was improved 10 to 100% by providing intermittent light, at 0.067 Hz at 50% duty ratio and 133 Hz at 50% duty ratio, respectively over continuous light.

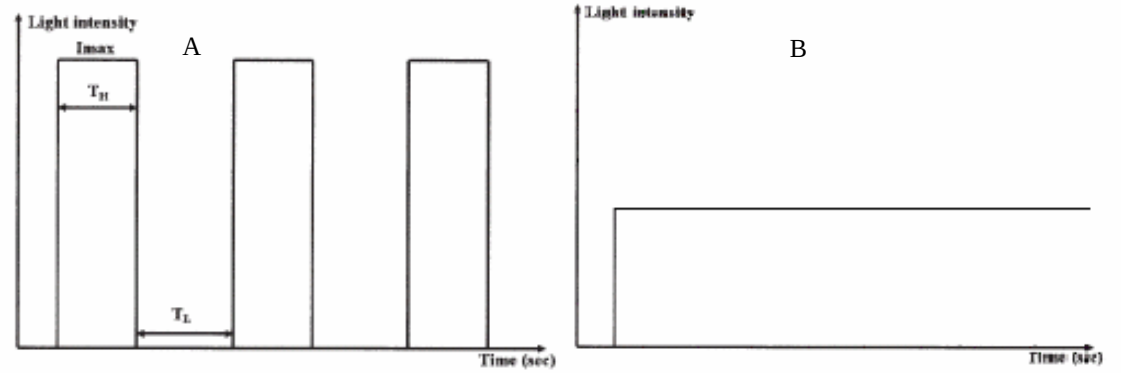

Figure 1.7. Intermittent light (A) versus continuous light (B). TH and TL mean the light on and off durations, respectively (Modified from Jao and Fang, 2004).

Emerson and Arnold (1932) observed a 400% increase in yield of photosynthetic oxygen per amount of light when *Chlorella pyrenoidosa* were illuminated at 50 Hz and 17% duty ratio. On the other hand, an improvement of yield in lettuce production was not observed using high frequency (37 kHz) fluorescent lamps compared with regular (60 Hz) fluorescent lamps (Hashimoto et al., 1988).

A different approach in studying intermittent light was adopted by Tennessen et al. (1995). They used LEDs to study the effect of light pulses (µs to ms) on photosynthesis of intact tomato leaves. The light response of photosynthesis was measured in continuous light and compared with

the same total photon flux but given in pulses that only lasted 1% of the time. They found that photosynthetic output was similar during light pulses and continuous light (50 µmol m^{-2} s^{-1}) treatments. However when light/dark pulses where lengthened to 2 ms of light and 198 ms of dark, net photosynthesis was reduced to half that measured in continuous light. Their conclusion was that plants use intermittent light (in kHz frequencies) only as good as they use continuous light. Emerson and Arnold (1932) found that at 25 °C, a dark period of 40 ms was "adequate for the complete removal of the material remaining at the end of each light flash." The same reason can be used to explain why the light/dark pulses lengthened to 2 ms of light and 198 ms dark reduced the net photosynthesis as observed by Tennessen et al. (1995) (Jao and Fang, 2004), because the dark period in this experiment was too long compared to the light period.

Sager and Giger (1980) analysed the published data of 14 experiments on intermittent light, to the extent possible, by a method introduced by Weller and Franck (1941), in which intermittent and continuous light are reduced to a common energy or photon flux density (independent variable) and the photosynthetic rate (dependent variable) is compared between light modes. Of the 14 experimental studies reviewed, consisting of both algal and higher plant experiments, the data of eight, but not necessarily the conclusions of the authors, supported the hypothesis of Rabinowitch (1956). This hypothesis was as follows; the rate of photosynthesis under intermittent light can approach but not exceed the rate under continuous light, for equal amounts of light applied during equal times under identical environmental conditions. One study did not support this hypothesis and five did not contain enough information to test the hypothesis. Therefore it seems like the hypothesis stated by Rabinowitch (1956) is true, or is at least in the right direction. This would mean that any research leading to a different outcome would be incorrect.

1.4 Research objective

The research objective for this research, which is specified by everything described above, is as follows.

To test the feasibility of using red LEDs in a pulsed light system as a light source for the growth of plants, by looking at the growth and photosynthetic parameters of these plants.

To investigate this several research questions were made.

- Is it possible to use pulsed LED light for plant growth?
- What are the effects of pulsed light on the growth of plant species?
- What is the cause of the effects of pulsed light on the growth of plant species?
- What are the operational limits of pulsed light if the normal growth of the plants has to be maintained?

The hypothesis is based on the one formulated by Rabinowitch (1956); the rate of photosynthesis and thus the growth of plants under intermittent light can approach but not exceed the photosynthesis rate and thus the growth under continuous light, for equal amounts of light applied during equal times under identical environmental conditions.

2. MATERIALS AND METHODS

2.1 Design of Spirodela trial experiment

The first experiment that has been set-up was used as a trial experiment, wherein it could be tested whether or not the duckweed fronds would grow under red light supplied by the LED arrays. Furthermore this first experiment was also used to check what kind of environment would be best to grow the duckweed fronds. For this experiment *Spirodela polyrhiza* was used, the fronds were taken from the 'stadsgracht of Wageningen' and they were allowed to grow for four days in a little container filled with water from the 'stadsgracht', replaced by demineralised water if water had evaporated. After this period the experiment was set up as a complete randomised design, there were 5 treatments; 24 hours or 16 hours of light, complete nutrient solution or 50% diluted nutrient solution, and red LEDs or fluorescent light (table 2.1). Each treatment was placed in a two litre plastic container, on which a floating raster was placed, wherein the *Spirodela* were placed (figure 2.1). The climate chamber that was used was set at 21 °C and 60% RH. The light intensity of each treatment was set at ± 200 µmol m^{-2} s^{-1}; however some variation was measured (table 2.2). Later on *Lemna minor* L. was also tested, however since it grew quite well in all the tested environments no data is discussed here.

Table 2.1. Treatment scheme for the first Spirodela experiment.

	Light colour [1]	Photoperiod (H)	Nutrients
1	Red	16	Complete
2	White	16	Complete
3	White	24	Complete
4	White	16	50% diluted
5	White	24	50% diluted

[1] Only the red colour is provided with LEDs the other ones are provided with Philips white fluorescent tubes of 58 Watt (TLD 84).

Figure 2.1. Placement of the Spirodela in the two litre plastic container

Table 2.2. Light intensity and variation therein of each treatment.

Treatment[1]	Light intensity ($\mu mol\ m^{-2}\ s^{-1}$)	Variation in light intensity ($\mu mol\ m^{-2}\ s^{-1}$)
1	195	9.0
2	197	5.5
3	201	2.5
4	191	10.0
5	193	7.0

[1] For the explanation of the treatment numbers see table 2.1

The contents of the nutrient solutions are given in appendix A. Any water which had evaporated was replaced on a daily basis with demineralised water, so that the distance between either the LED array or the fluorescent tubes and the aquarium remained the same at all times. This meant however, that the amount of nutrients in the solution dropped over time, because of the nutrients taken up by the *Spirodela* fronds themselves.

Measurements done on the trial experiment.

Photos were taken from every treatment on a daily basis and analysed by using ImageJ (ImageJ version 1.32J, Wayne Rasband, National Institute of health, USA), further explained in the next paragraph, 2.2. After this analysis it was possible to check how the growth of the *Spirodela* fronds proceeded when looking at the area occupied by the *Spirodela* fronds.
On every Monday, Wednesday and Friday one cube, from the raster, of each treatment was taken out and the fresh and dry weight was measured. The dry weight ratio was also calculated to see if this was influenced. The dry weight was established by placing the *Spirodela* fronds in an oven located at Unifarm (appendix B).

2.2 Lemna experiments

Choice for Lemna

In the rest of the experiments *Lemna minor* L. was chosen over *Spirodela polyrhiza* Scheid. both are duckweed species; however the *Lemna* as its name already suggests is quite small. This made it easier to grow them in the nutrient solution without self shading etc. The main reason why duckweed was used as a model plant in this experiment is just that, that it is a widely used model plant. When the first physicists started with experiments on photosynthesis they used *Lemna* as a model plant, primarily because you only have to deal with one layer of leaves, so the processes that occur are easy to follow. Moreover, there is a lot of information available on the growth and development of duckweed. But perhaps most importantly, *Lemma* is a relatively easy plant to grow; it will grow in almost any environment and won't ever suffer from water stress, since it is an aquatic plant. It remains quite small, which is also essential for this experiment, since the LED arrays constructed where only 500 cm^2. With this area it is very difficult to give light to plants, since most plants that grow quickly, like *Lemna* does, quickly exceed this area. Whereas *Lemna* is able to grow quite fast, a growth curve can be estimated in approximately nine days, but remains small enough to be kept under these arrays. Duckweed species are higher plants, and they react in substantially the same way as other higher plants do, therefore it is acceptable to extrapolate the conclusions on duckweed to other plant species.

Set-up of the Lemna experiments

All of the experiments on the *Lemna* fronds were conducted in a similar manner, in the same climate chamber and each of the treatments constantly placed at the same position within the climate chamber (appendix C). The climate chamber had as set points a temperature of 21 °C and a relative humidity of 60%, relatively no variation was detected in the temperature; however the relative humidity was often higher but never lower than the set point. The carbon dioxide concentration within the chamber remained at ambient levels.

For the cultivation of duckweed, 11.5 X 11.5 X 18 cm plastic aquaria containing two litres of growth media were used. The set-up within an aquarium is essentially the same as for the *Spirodela* experiment (figure 2.1). The growth medium used was a standardized tomato nutrient solution (provided by Unifarm), which was diluted by 50% with demineralised water. An analysis of the amount of nutrients present in this medium is given in appendix A. Every day the water

that had evaporated was replaced with demineralised water, also every day after this water was added the pH and the EC of the solutions was measured (WTW pH/Cond 340i), so that any variations resulting from differences in these factors could be explained. Hardly any differences in the pH was measured, but over the different experiments the EC differed very much, but not between treatments within an experiment (data not shown).

A nursery containing the duckweed fronds was set up shortly after taking the fronds out of a ditch, so that the fronds used for each experiment always came out of a similar background. This nursery was also placed in the climate chamber and placed at approximately the same conditions as the actual treatments, so that the variation in non test conditions was as low as possible. The fronds in the nursery were kept in an exponential growth stage, by removing some fronds when it became overcrowded. Any fronds that did not look healthy, for example because of chlorosis or necrosis, were also removed. When algal or microbial contaminations were noticed on the fronds, they were also taken out.

The daily light integral for each treatment was always set at 11.52 mol m^{-2} day^{-1}. For the fluorescent treatment this was achieved by placing the aquarium containing the *Lemna* fronds on a 'labjack', that was able of altering the distance between the fluorescent tubes and the aquarium, in that way altering the light intensity that reached the fronds. For the LED treatments this was achieved by altering the current over the array, and thus decreasing/increasing the light intensity provided by the arrays.

The photoperiod was set at 16 hours, predominantly because the fluorescent tubes were already installed in this way, achieved by cutting off the current to the LED arrays (see paragraph 6.2 for details).

The duckweed fronds were placed in a floating raster with 12 separately confined cubes; so that every treatment had 12 separate repetitions (figure 2.1). Before they were placed in the nutrient solution, they were subjected to a method to reduce the amount of microbial and algal activity. This method was based on a method used by Chua and Dickson (1964), and consisted of rinsing the fronds in demineralised water, dipping them in 80% ethanol followed by a few seconds emersion in 1% sodium hypochlorite (bleach) and subsequent rinsing in demineralised water, and finally placing them in the appropriate cube within the raster. This method resulted in some growth retardation of the duckweed fronds; however the resulting reduction in algal and microbial activity was more beneficial. When all of the fronds had been placed in the three aquaria, a treatment was randomly placed under a light source.

Measurements done on Lemna

The following measurements were conducted on the *Lemna* fronds, and they will be discussed below; relative area growth rate, photosystem II efficiency, dry weight and red, green and blue (RGB) values of the fronds.
The measurement of the relative area growth rate was done in a way described by Evans (1972). The formula he describes is slightly modified here to represent area growth rate.

$$A_n = A_0 * e^{R*(T_n - T_0)} \quad \text{(eq 2.1)}$$

With:
A_n = area present at time n,
e = base natural logarithm (2.718),
R = mean relative area growth rate,
T_n = time at n

So to get the mean relative area growth rate of a treatment it is necessary to know the area that is present on a daily basis, so that this formula is based on as much data as is possible. Therefore every day a photo was taken of every treatment. From these photos the area that was present was calculated with an analysis done in ImageJ (version 1.32J, Wayne Rasband, National institute of health, USA). The picture was analysed per cube, the picture was centred and enlarged for every cube consecutively. Around the duckweed fronds some white distortions regularly occurred, these where filtered out. After this the centred picture was split in three different pictures; the red, green and blue part of the original picture, respectively. The green part was then thresholded, so that only the area of the fronds was converted to black and the rest of the picture remained white. The black area that was the result of these steps was then analyzed, so that the area in pixels was measured. This area in pixels was converted with a known area in pixels, measured with a coin, into an area in square centimetres. The area in square centimetres was then used over a period of ten days to fit the exponential growth formula (eq 2.1), this was done with Microsoft excel (version 10.5815.4219 SP2, Microsoft corporation, 2002), from this fitted equation the mean relative area growth rate (R in eq 2.1) per cube could be read. However, since the fronds were treated with sodium hypochlorite and where moved to a different environment, it took two days before they reached a state of exponential growth. This meant that these first two days were kept out of the calculations of the mean relative area growth rate. The values for the mean relative area growth rate where then analysed by an analysis of variance test in Genstat (Version 7.1.0.205, Lawes agricultural trust, 2003).

At the end of each experimental period the aquaria containing the duckweed fronds were placed under a chlorophyll fluorescence imaging system (FluorCam) (Photon Systems Instruments (700 MF, SN: FC8080)), except for experiment 2, in that experiment this fluorcam was not available, so another fluorcam (Photon Systems Instruments (700 MF, SN: FC8030)) was used. This was done to estimate the quantum yield of photosystem II (ΦPSII), calculated using the following equation (Rosenqvist and Kooten, 2003):

$\Phi PSII = \Delta F' / F_m' = (F_m' - F_s') / F_m'$ (eq 2.2)

With:

F' = fluorescence

F_m' = maximal fluorescence

F_s' = steady state fluorescence

This quantum yield was estimated for multiple actinic light percentages; 0% (dark adapted fluorescence), 20%, 60% and 100%. Corresponding to a light intensity at the height of the *Lemma* of 0, 6, 18 and 30 µmol m^{-2} s^{-1} respectively. Before the fluorescence image was taken, the aquaria where adapted to the corresponding actinic light intensity for 20 minutes. This was done to acclimate the plants and PSII to the light intensity used.

After the fluorescence imaging was completed, all of the samples were taken out and put into an oven to dry (appendix B). When the samples where dry, they were weighed and the so obtained dry weight was analysed with an analysis of variance test in Genstat. Furthermore the leaf area ratio (LAR) was calculated from the following equation (Evans, 1972).

$$LAR = \frac{A_L}{W} \quad \text{(eq 2.3)}$$

With:

A_L = leaf area, in this case frond area

W = total dry weight

The LAR was then analysed by an analysis of variance test in Genstat (Version 7.1.0.205, Lawes agricultural trust, 2003). This particular index has been chosen over the specific leaf area, which in principle is a more accurate index, because it is nearly impossible to get separate weights of the roots and the 'leaves' in duckweed, and that these separate weights are needed for that index.

The dry weight of the plants on day 0 was also calculated as the area present at day 0 multiplied by the LAR of the continuous fluorescent treatment at the end of the experiment. This could be done because the continuous fluorescent treatment was essentially the same as the nursery from

where the fronds where taken out of. This calculation was made because the differences in dry weight at the end of the experiment could be explained by differences in the beginning of the experiment.

The RGB values of the different fronds were measured from the photos taken on the last day of each experiment; this was done because the greenness of a frond, or leaf for that matter, says something about the chlorophyll content of the frond, which says something of the radiation absorption capability of the fronds. Because a lot of the fronds were growing under an angle to the photo camera a lot of ‘highlights’ occurred. These ‘highlights’ did not have the same RGB-values as the normal fronds; therefore only small spots of fronds per cube could be measured. Furthermore it was not possible to compare these values between different experiments. This was caused by the ‘background lighting’, by which it is meant that the light levels inside the climate chamber where not always the same between different experiments, mainly because of other experiments going on inside the climate chamber. It was possible however to compare between different treatments within one experiment. The results for the different treatments did not differ much, however the results within one treatment and even within one cube within a treatment differed considerably, and therefore these results are not taken into account any further.

2.3 Pulsing experiments

The pulsing of the light output was done with a standardized oscillator, which was only capable of altering the frequency of the signal. Thus the duty ratio of each experiment always remained 1, which means that the 'on'-signal was as long as the 'off'-signal. Also in the first two experiments, as well as in the first three days of the third experiment the box that turned the signal completely off was not yet finished, so the light intensity of the 'off'-signal was still 27.5 µmol m^{-2} s^{-1}. This was taken into account when setting up the daily light integral, in that the 'on'-signal was lower, however in the first experiment an error was made and the 'on'-signal was higher than thought. All light intensities were measured with a quantum sensor (Licor LI-250). Each treatment was always checked against two treatments conducted at the same time, of which one was a continuous red LED system, and the other one consisted of continuous white fluorescent tubes of 58 Watt (Philips TLD 84). Both treatments had the same daily light integral as the pulsed LED system, except for experiment 1, as already discussed above. The experiments conducted had a frequency ranging from 0.012 till 120 Hz with steps of a factor ten. This would result in five experiments, but again as already discussed above, the experiment at 1.28 Hz was repeated because of some errors. The first time it was conducted is still described in this report because it shows the effect that the daily light integral has. The different experiments are numbered according to the time when they were performed, so experiment 1 was done first, followed by 2 etc. (table 2.3).

The light intensity of each treatment was measured at the beginning of experiment 3, except for the pulsed LED treatment, which was measured halfway this experiment, since the 'box' that turned the signal totally off was installed then, and at the end of experiment 6 (table 2.4). All light intensities were measured with a quantum sensor (Licor LI-250).

At the end of experiment 6 a system was also set-up which could measure the daily light integral (DLI), this was done to check the calculated DLI. The system consisted of a photodiode that was linked to a computer, which served as a data logger. The photodiode was first used in combination with the quantum sensor, to calculate a formula which would relate the light intensity to the output of the photodiode (eq 2.4).

Light intensity (µmol m^{-2} s^{-1}) = - 90.653 * photodiode output + 4.969 (eq 2.4)

However some strange data points occurred, for example when there should be no signal at all, so a complete dark environment, the calculated light intensity from the output of the photodiode was still 5 µmol m^{-2} s^{-1}, this has a huge influence on the daily light integral, because with a dark

period of 8 hours, this results in an overestimation of the DLI of 144300 µmol. And there will probably be some more deviations throughout the 24 hours. So the data have to be taken with a 'korrel of zout' as we say in Dutch, which means that we should not believe everything that the data says (table 2.4).

Table 2.3. Overview of the Lemna pulsing experiments conducted.

Experiment	Frequency (Hz)	Light intensity (µmol m^{-2} s^{-1})		Daily light integral (mol m^{-2} day^{-1})
		'off'-signal	'on'-signal	
1	1.28	27.5	400	12.31
2	1.28	28	372	11.52
3	0.12	0	400	11.52
4	12	0	400	11.52
5	0.012	0	400	11.52
6	120	0	400	11.52

Table 2.4. Light intensity and the variation therein and the measured daily light integral of the different Lemna treatments.

Treatment	Light intensity (µmol m^{-2} s^{-1})		Variation in the light intensity (µmol m^{-2} s^{-1})		Daily light integral (mol m^{-2} day^{-1})
	Beginning[1]	End[2]	Beginning[1]	End[2]	
Continuous fluorescent	200	195	20	10	12.32
Continuous LED	200	184	5	12	13.18
Pulsed LED	400	377	10	17	13.51

[1] Beginning means the beginning of experiment 3, except for pulsed LED as discussed
[2] End means at the end of experiment 6

3. RESULTS

3.1 Spirodela trial experiment

There were no significant differences (P<5%) between the two lighting periods (P=0.146) and between the two nutrient treatments (P=0.973), when looking at the average area in square centimetre occupied by the *Spirodela* fronds (table 3.1 and appendix D). Also for the dry weight of the fronds, there is no significant difference between the different treatments (P=0.342 and P=0.677 respectively) (table 3.2 and appendix D); however this was mainly caused by a very high variability within treatments. Since there were also large differences in dry weight, for example the treatment with complete nutrient solution and a photoperiod of 16 hours weight less than half of the same nutrient treatment at a photoperiod of 24 hours, 0.51 and 1.18 mg respectively.

Table 3.1. Statistical analysis of the effect of different nutrient and light period treatments on the average area (cm^2) of the Spirodela fronds.

Light period	Complete nutrients	Half nutrient half demi-water
16 hours	1.94 NS[1]	2.15 NS
24 hours	3.02 NS	2.86 NS

[1] NS means that there is no significant difference (P<5%).

Table 3.2. Statistical analysis of the effect of different nutrient and light period treatments on the dry weight (mg) of the Spirodela fronds.

Light period	Complete nutrients	Half nutrient half demi-water
16 hours	0.51 NS[1]	0.63 NS
24 hours	1.18 NS	0.73 NS

[1] NS means that there is no significant difference (P<5%).

If the average area in square centimetre and the dry weight of the fronds under the different light sources with the same nutrient treatment and light period, complete nutrient solution and 16 hours respectively, are compared then there is no significant difference (P=0.284 and P=0.932 respectively) between light from the LED array or from the fluorescent tubes (table 3.3, table 3.4 and appendix D).

Table 3.3. Statistical analysis of the effect of the different light sources on the average area (cm^2) of the Spirodela fronds.

Light source	Average area
Fluorescent	1.94 NS[1]
LED	1.41 NS

[1] NS means that there is no significant difference (P<5%).

Table 3.4. Statistical analysis of the effect of the different light sources on the dry weight (mg) of the Spirodela fronds.

Light source	Dry weight (mg)
Fluorescent	0.51 NS[1]
LED	0.54 NS

[1] NS means that there is no significant difference (P<5%).

3.2 Pulsing experiments

The intensity of the wavelength of the pulsed LED treatment was much higher then for the continuous LED treatment; this was caused by the fact that the pulsed LED treatment had a light intensity that was two times higher than that of the continuous LED treatment (table 2.4). The wavelength of the fluorescent treatment consisted primarily of peaks at the blue, red and green part of the spectrum, what is interesting however is that the peak in the red is at a slightly lower wavelength than that of the LEDs. Furthermore a lot of the energy provided by the fluorescent lamps is less efficiently utilized by the *Lemna* because the peak at approximately 550 nm coincides with the lowest point in the absorption spectrum of leaves as shown in figure 1.1 (figure 3.1).

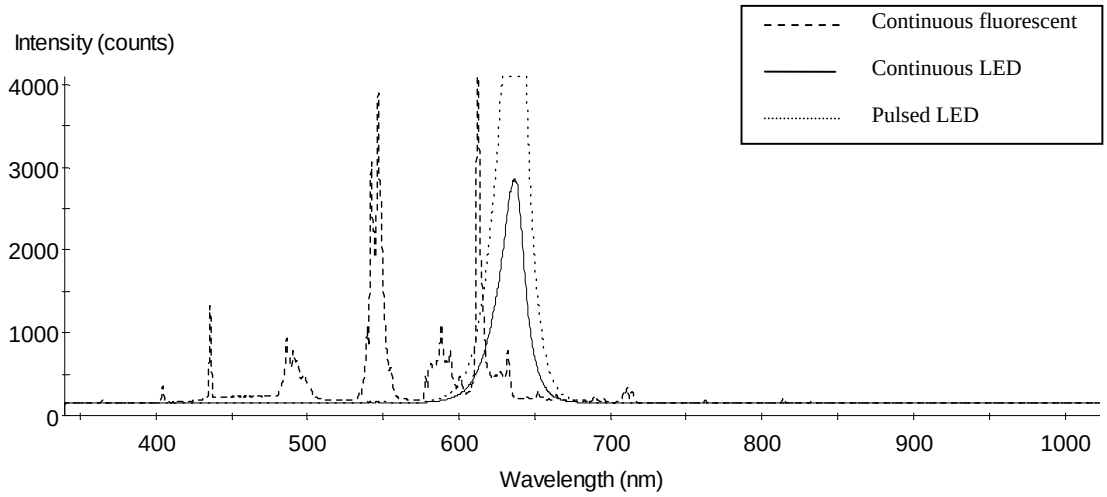

Figure 3.1. The wavelength of the light sources used for the different Lemna treatments. The continuous and pulsed LED systems have the same wavelength; the pulsed LED is only higher because of the higher light intensity of the pulsed treatment (table 2.4) and it is actually much higher then shown here. This is caused because with this graph the scale only goes to 4000, but the pattern is the same as for the continuous treatment. The fluorescent system consists primarily of peaks in the red, green and blue.

The area growth of the duckweed fronds between each measurement, averaged over the 12 cubes, was plotted against time. This mostly resulted in a distinct pattern of acclimation to the environment in the first two days and then a ‘sinusoide’ type of function, of a day, or several days of high growth, followed by a day, or several days, of lower growth. This pattern occurred for all the treatments and for all of the experiments, and an example is shown in figure 3.2, this is the figure of the first experiment, at 1.28 Hz.

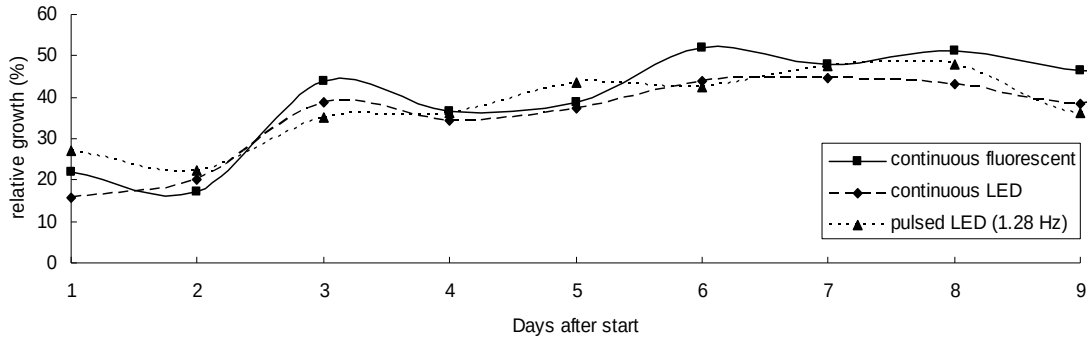

Figure 3.2. An example of a plot of the relative growth (%) of the different Lemna treatments between each measurement. This shows the distinct patterns in the relative growth, in that the first few days the plants need to acclimate to their environment, and after that show a pattern of ups and downs. This plot is of the first 1.28 Hz treatment.

To eliminate the pattern in the relative growth (figure 3.2), the area occupied by the Lemna in each cube was plotted against time and then an exponential growth curve was fitted on this data, to get a measure of the relative area growth rate (paragraph 2.2). When plotting the exponential growth curve, the first two days are left out, because of the acclimation period of the duckweed plants. In figure 3.3 this is shown for the first 1.28 Hz treatment, for the continuous LED treatment in part A and for the pulsed LED treatment in part B. In both parts the highest and lowest growth curves are plotted. In the continuous LED treatment the R^2, which is a measure for how good the fit aligns with the data points, for the highest and the lowest growth curve are 0.9972 and 0.9881, respectively. For the pulsed LED treatment these figures are 0.999 and 0.992, respectively. For both treatments the figures mentioned are very high, which means that the fit is very good. This is in general the case with the fits of the exponential growth curve.

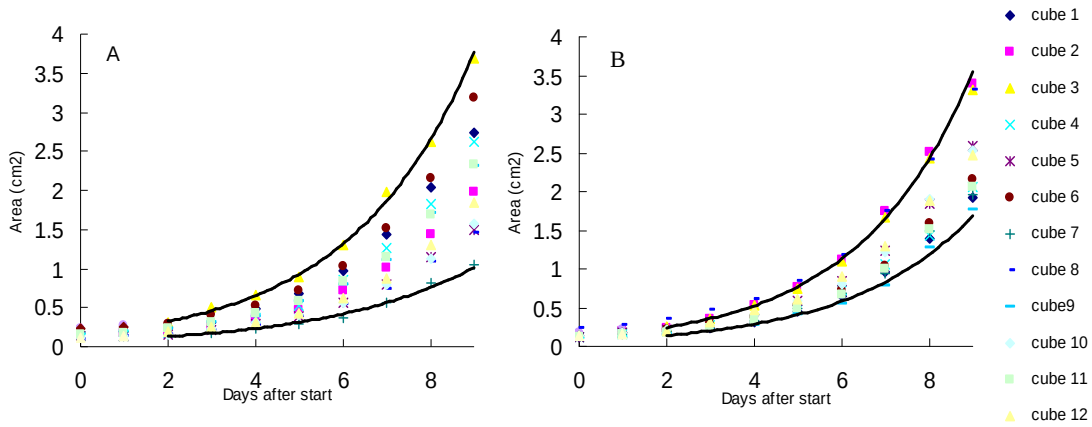

Figure 3.3. An example of the graphs showing the area occupied by the duckweed fronds in each cube, which was used to estimate the exponential growth formula. In part A the continuous LED treatment is shown and in part B the pulsed LED treatment is shown. These data were taken from the first experiment at 1.28 Hz. In both parts the highest growth curve and the lowest growth curve are fitted.

Experiment 1

Experiment 1 was the first experiment at 1.28 Hz. The dry weight of the duckweed fronds was calculated for day 0 as area*leaf area ratio of the continuous fluorescent treatment, since this was expected to be roughly the same as that of the nursery. There was no significant difference (P=0.458) between the different treatments. The dry weight of the duckweed fronds was measured at the end of the experiment and there was no statistical difference (P<5%) between the different treatments (table 3.5 and appendix E), however the probability was very low (P=0.073). The dry weight measurement was based on 11 of the 12 cubes because of a continuation experiment with the last cube, however. Also this measurement was taken after ten days, whereas the relative area growth rate sampling stopped after nine days. The relative area growth rate of the different treatments differed significantly (P<0.001). The duckweed fronds under the fluorescent light had the highest relative area growth rate, the continuous LED treatment the lowest and the pulsed LED was in between (figure 3.4 and appendix E). These data were based on 12 samples per treatment over 9 days. When looking at the leaf area ratio (LAR), so the area divided by the dry weight, than there is no significant difference between the continuous fluorescent and the pulsed LED treatment, and both have a significantly higher LAR than the continuous LED treatment (P<0.001) (figure 3.5 and appendix E). However the area measurement was at day nine and the dry weight measurement at day ten, so this could give some strange results.

Table 3.5. Statistical analysis of the effect of the different treatments on the dry weight (mg) of the Lemna fronds and a comparison with the dry weight at the start of the experiment.

Treatments	Dry weight (mg)	
	Day 0	Day 10
Continuous fluorescent	0.049[1] NS[2]	0.942 NS[2]
Continuous LED	0.045 NS	0.778 NS
Pulsed LED (1.28 Hz)	0.043 NS	0.715 NS

[1] Calculated as the area of the duckweeds present at day 0 * leaf area ratio of the continuous fluorescent treatment.
[2] NS indicates that there is no significant difference within columns (P<5%).

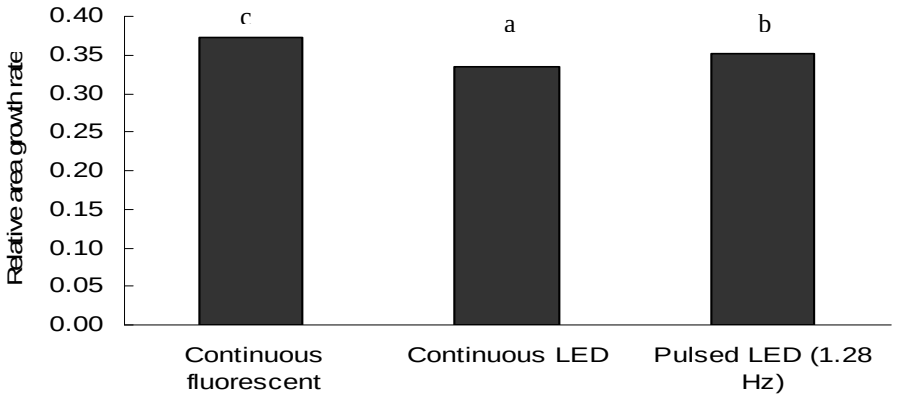

Figure 3.4. Relative area growth rate (cm^2 cm^{-2} day^{-1}) of the various Lemna treatments. Different letters above the bars indicate significant (P<5%) differences as established by the LSD-test. LSD = 0.01689.

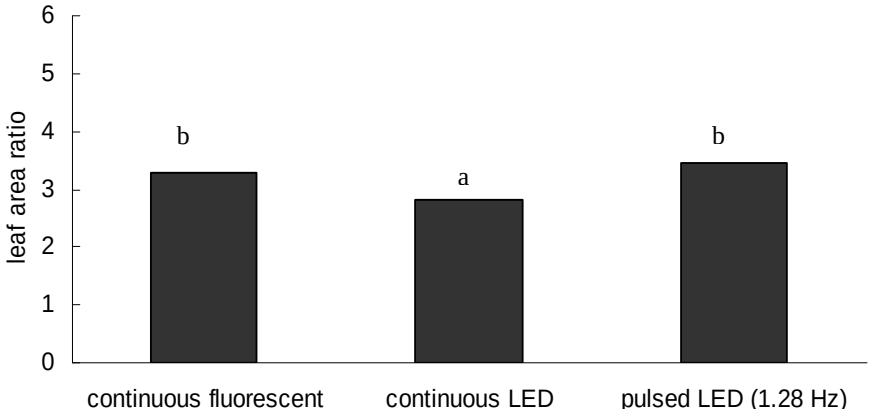

Figure 3.5. LAR (cm^2 mg^{-1}) of the various Lemna treatments. Different letters above the bars indicate significant (P<5%) differences as established by the LSD-test. LSD = 0.2642.

The average relative PSII efficiency over the different treatments was calculated and this showed that both LED treatments have a slightly lower efficiency as the fluorescent treatment, where the pulsed treatment is lower then the continuous treatment, except for 0% actinic light, where the pulsed LED treatment had the highest efficiency. Furthermore when the actinic light intensity is increased, the efficiency decreases, not by much however, and at 100 % actinic light the efficiency is higher again, than at 60% actinic light (figure 3.6).

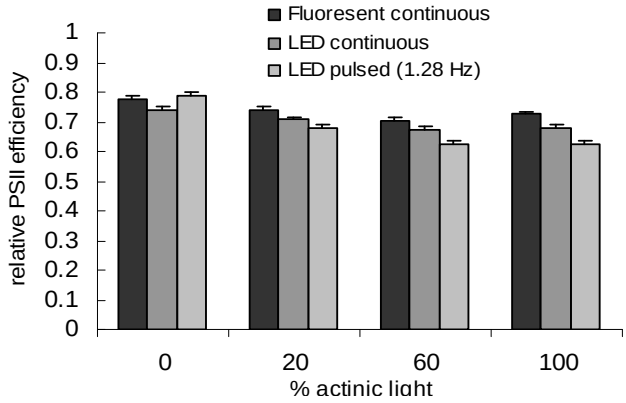

Figure 3.6. Average relative photosystem II efficiency over each treatment as established by chlorophyll fluorescence, error bars indicate standard deviations.

Experiment 2

Experiment 2 was the second experiment at 1.28 Hz; this was repeated because the daily light integral of the pulsed treatment was too high in the previous experiment. The dry weight of the duckweed fronds on day 0 was calculated, there was no significant difference (P=0.642) between the different treatments. The dry weight of the duckweed fronds was measured at the end of the experiment and there was no statistical difference (P=0.880) between the different treatments (table 3.6 and appendix E).

Table 3.6. Statistical analysis of the effect of the different treatments on the dry weight (mg) of the Lemna fronds and a comparison with the dry weight at the start of the experiment

Treatments	Dry weight (mg)	
	Day 0	Day 9
Continuous fluorescent	0.028[1] NS[2]	0.400 NS[2]
Continuous LED	0.031 NS	0.418 NS
Pulsed LED (1.28 Hz-2)	0.030 NS	0.416 NS

[1] Calculated as the area of the duckweeds present at day 0 * leaf area ratio of the continuous fluorescent treatment.
[2] NS indicates that there is no significant difference within a column (P<5%).

The relative area growth rate of the different treatments differed significantly (P<0.001). The duckweed fronds under the fluorescent light had the highest relative area growth rate, the continuous LED treatment the lowest and the pulsed LED was in between (figure 3.7 and appendix E). When looking at the LAR, than there is no significant difference between the continuous fluorescent and the pulsed LED treatment, and both have a significantly higher LAR than the continuous LED treatment (P=0.005) (figure 3.8 and appendix E).

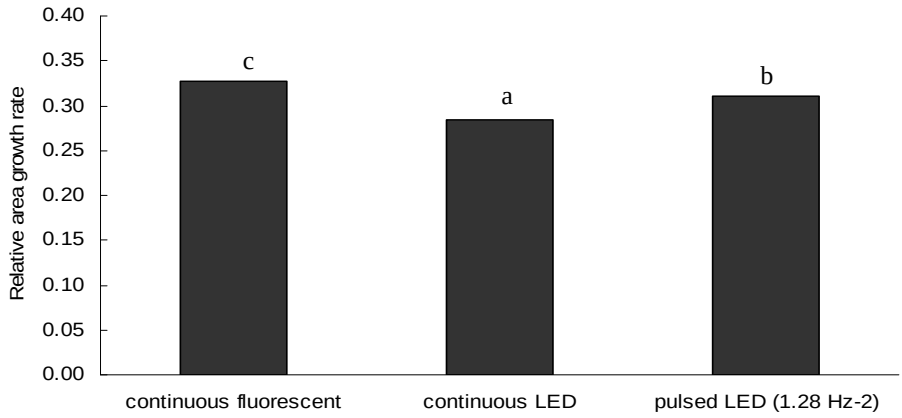

Figure 3.7. Relative area growth rate (cm^2 cm^{-2} day^{-1}) of the various Lemna treatments. Different letters above the bars indicate significant (P<5%) differences as established by the LSD-test. LSD = 0.01604.

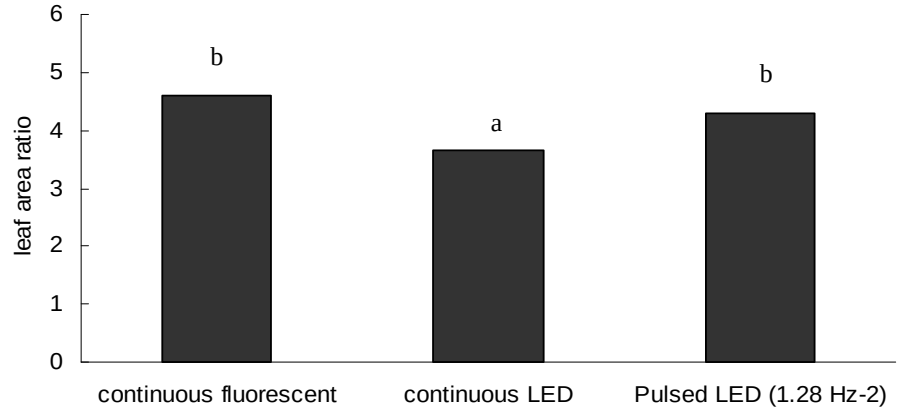

Figure 3.8. LAR (cm^2 mg^{-1}) of the various Lemna treatments. Different letters above the bars indicate significant (P<5%) differences as established by the LSD-test. LSD = 0.554.

The average relative PSII efficiency over the different treatments was calculated and this showed that there was not much variation between the different treatments; however the pulsed LED treatment was usually lower. Because of some technical constraints the 100% actinic light

pictures were not taken. Furthermore when the actinic light intensity is increased, the efficiency decreases, not by much however. The higher standard deviation in this figure compared to other figures (for example figure 3.6) was caused by the fact that these data were taken with an older Fluorcam because the other one was in use (figure 3.9).

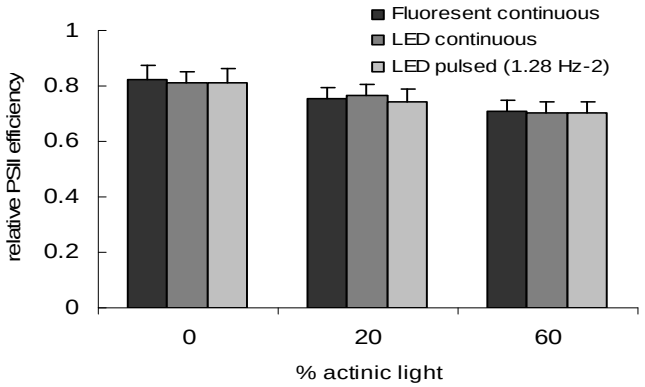

Figure 3.9. Average relative photosystem II efficiency over each treatment as established by chlorophyll fluorescence, error bars indicate standard deviations.

Experiment 3

In experiment 3 the frequency of the pulses was 0.12 Hz; this frequency was chosen because of the factor ten differences between this one and the previous ones. The dry weight of the duckweed fronds on day 0 was calculated, there was no significant difference (P=0.502) between the different treatments. The dry weight of the duckweed fronds at the end of the experiment was measured and the two continuous treatments, fluorescent tubes and LEDs, had a significantly higher (P<0.001) dry weight than the pulsed LED treatment (table 3.7 and appendix E).

Table 3.7. Statistical analysis of the effect of the different treatments on the dry weight (mg) of the Lemna fronds and a comparison with the dry weight at the start of the experiment

Treatments	Dry weight (mg)	
	Day 0	Day 9
Continuous fluorescent	0.030[1] NS[2]	0.516 b[3]
Continuous LED	0.029 NS	0.598 b
Pulsed LED (0.12 Hz)	0.032 NS	0.379 a

[1] Calculated as the area of the duckweeds present at day 0 * leaf area ratio of the continuous fluorescent treatment.
[2] NS indicates that there is no significant difference within a column (P<5%).
[3] Means followed by different letters differ significantly (*P*<5%), within a column, as established by the LSD-test. LSD = 0.0846

The relative area growth rate of the different treatments differed significantly (P<0.001). The duckweed fronds under the fluorescent light and under the continuous LED light had a significantly higher relative area growth rate than the fronds under the pulsed LED light (figure 3.10 and appendix E). When looking at the LAR, than the pulsed LED treatment has a significantly higher LAR then the continuous LED treatment, and both do not differ significantly from the continuous fluorescent treatment (P=0.025) (figure 3.11 and appendix E).

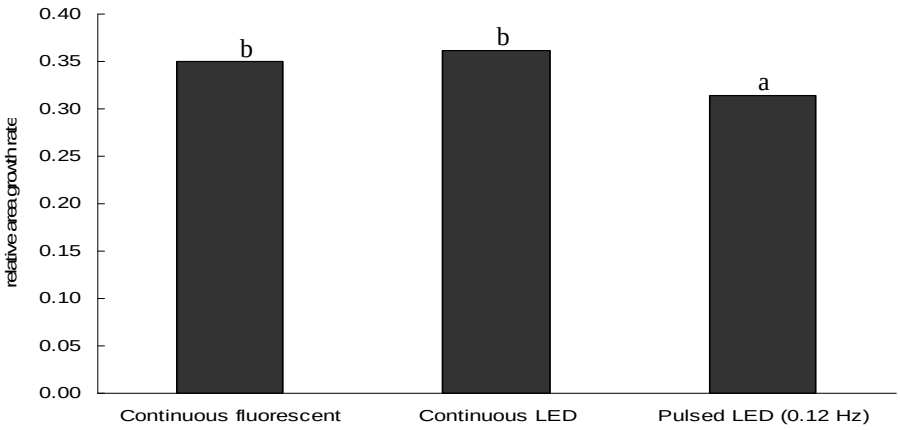

Figure 3.10. Relative area growth rate (cm^2 cm^{-2} day^{-1}) of the various Lemna treatments. Different letters above the bars indicate significant (P<5%) differences as established by the LSD-test. LSD = 0.01344.

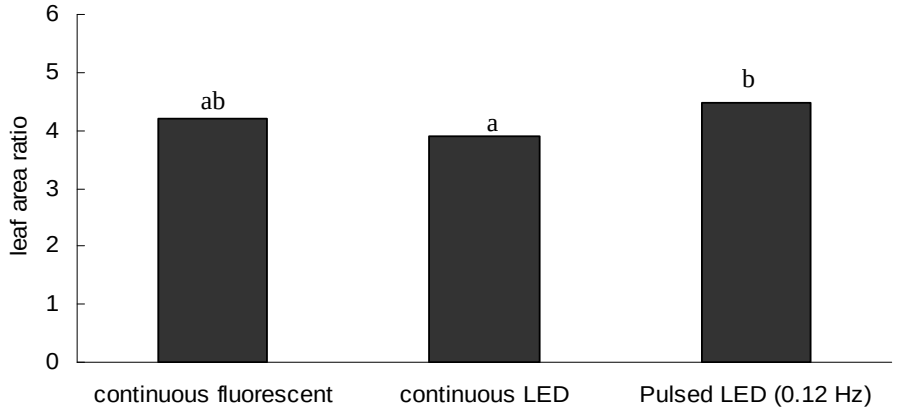

Figure 3.11. LAR (cm^2 mg^{-1}) of the various Lemna treatments. Different letters above the bars indicate significant (P<5%) differences as established by the LSD-test. LSD = 0.4106.

The average relative PSII efficiency over the different treatments was calculated and this showed that both LED treatments have a slightly lower efficiency as the fluorescent treatment, where the

pulsed treatment is lower then the continuous treatment. Furthermore when the actinic light intensity is increased, the efficiency decreases, not by much however, and at 100 % actinic light the efficiency is higher again than at 60% actinic light (figure 3.12).

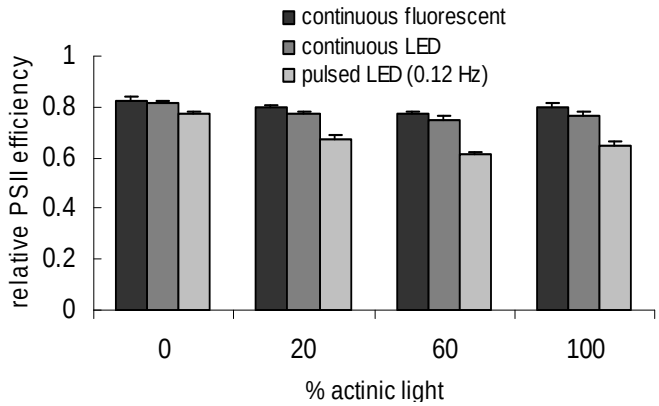

Figure 3.12. Average relative photosystem II efficiency over each treatment as established by chlorophyll fluorescence, error bars indicate standard deviations.

Experiment 4

In experiment 4 the frequency of the pulses was 12 Hz; this was chosen to be a factor ten different from the first experiment only to the other side as experiment 3. The dry weight of the duckweed fronds at the start of the experiment was calculated, the continuous fluorescent treatment had a significantly higher (P=0.013) dry weight then both LED treatments, continuous and pulsed. The dry weight of the duckweed fronds at the end of the experiment was measured and there was a statistical difference (P<0.001) between the different treatments (table 3.8 and appendix E), the continuous fluorescent treatment had the highest dry weight, followed by the continuous LED treatment and the pulsed LED treatment had the lowest dry weight.

Table 3.8. Statistical analysis of the effect of the different treatments on the dry weight (mg) of the Lemna fronds and a comparison with the dry weight at the start of the experiment

Treatments	Dry weight (mg)	
	Day 0	Day 9
Continuous fluorescent	0.026[1] b[2]	0.534 c[2]
Continuous LED	0.022 a	0.429 b
Pulsed LED (12 Hz)	0.021 a	0.344 a

[1] Calculated as the area of the duckweeds present at day 0 * leaf area ratio of the continuous fluorescent treatment.
[2] Means followed by different letters differ significantly (*P*<5%), within a column, as established by the LSD-test. LSD = 0.003253 for day 0 and LSD = 0.0867 for day 9.

The relative area growth rate of the continuous fluorescent treatment was significantly higher (P<0.001) then the other two treatments. There was no significant difference between the continuous LED and the pulsed LED treatment (figure 3.13 and appendix E). The continuous fluorescent treatment had a significantly higher (P<0.001) leaf area ratio (LAR) then the other two treatments. There was no significant difference between the continuous and pulsed LED treatments (figure 3.14 and appendix E).

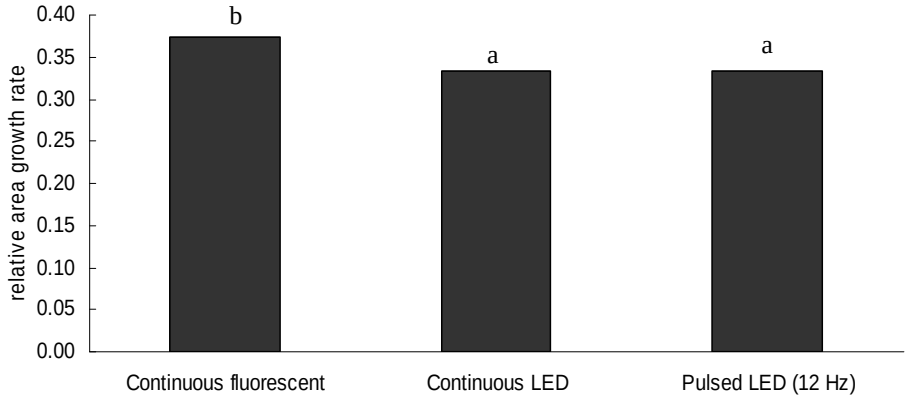

Figure 3.13. Relative area growth rate (cm^2 cm^{-2} day^{-1}) of the various Lemna treatments. Different letters above the bars indicate significant (P<5%) differences as established by the LSD-test. LSD = 0.01172.

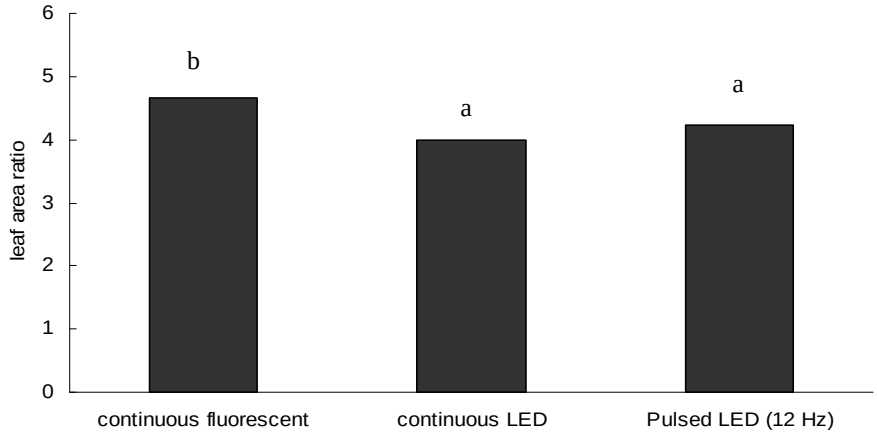

Figure 3.14. LAR (cm^2 mg^{-1}) of the various Lemna treatments. Different letters above the bars indicate significant (P<5%) differences as established by the LSD-test. LSD = 0.3258.

The average relative PSII efficiency over the different treatments was calculated and this showed that both LED treatments have a slightly lower efficiency as the fluorescent treatment, where the pulsed treatment is lower then the continuous treatment. Furthermore when the actinic light intensity is increased, the efficiency decreases, not by much however (figure 3.15).

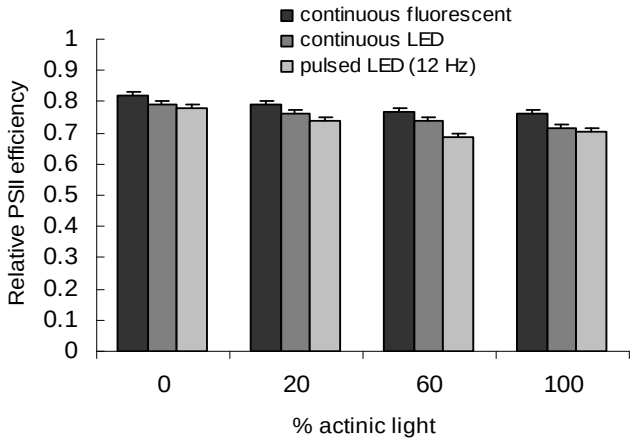

Figure 3.15. Average relative photosystem II efficiency over each treatment as established by chlorophyll fluorescence, error bars indicate standard deviations.

Experiment 5

In experiment 5 the frequency of the pulses was 0.012 Hz; this was chosen to see what would be the lowest possible frequency and this was a factor ten lower than experiment 3. The dry weight of the duckweed fronds at the start of the experiment was calculated, there was no significant difference ($P=0.135$) between the different treatments. The dry weight of the duckweed fronds on day 9 was measured and both continuous treatments, LEDs and fluorescent tubes, had a significantly higher ($P<0.001$) dry weight then the pulsed LED treatment (table 3.9 and appendix E).

Table 3.9. Statistical analysis of the effect of the different treatments on the dry weight (mg) of the Lemna fronds and a comparison with the dry weight at the start of the experiment

Treatments	Dry weight (mg)	
	Day 0	Day 9
Continuous fluorescent	0.038[1] NS[2]	0.884 b[3]
Continuous LED	0.042 NS	0.862 b
Pulsed LED (0.012 Hz)	0.037 NS	0.277 a

[1] Calculated as the area of the duckweeds present at day 0 * leaf area ratio of the continuous fluorescent treatment.
[2] NS indicates that there is no significant difference within a column (P<5%).
[3] Means followed by different letters differ significantly (*P*<5%) within a column, as established by the LSD-test. LSD = 0.1037

The relative area growth rate of the treatments was calculated and there was a significant difference (P<0.001), the continuous fluorescent treatment had the highest relative area growth rate, followed by the continuous LED treatment and the pulsed LED treatment had the lowest (figure 3.16 and appendix E). The pulsed LED treatment had a significantly higher (P<0.001) leaf area ratio (LAR) then the other two treatments. The other two treatments also differed significantly, in that the continuous LED treatment had a lower LAR then the continuous fluorescent treatment (figure 3.17 and appendix E).

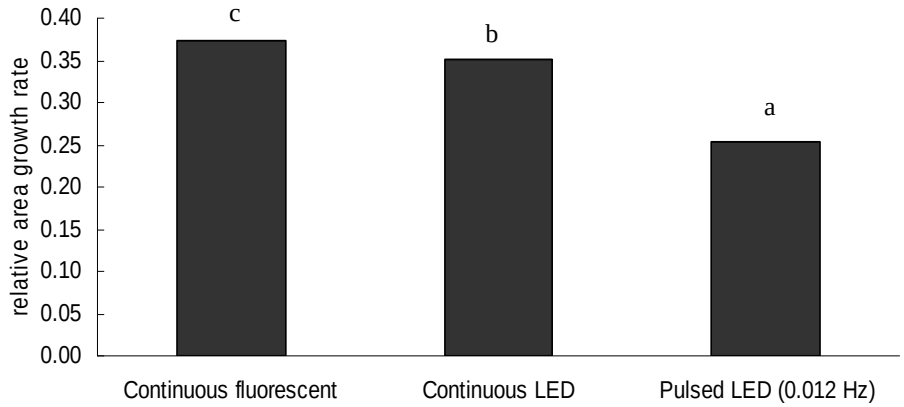

Figure 3.16. Relative area growth rate (cm^2 cm^{-2} day^{-1}) of the various Lemna treatments. Different letters above the bars indicate significant (P<5%) differences as established by the LSD-test. LSD = 0.00930.

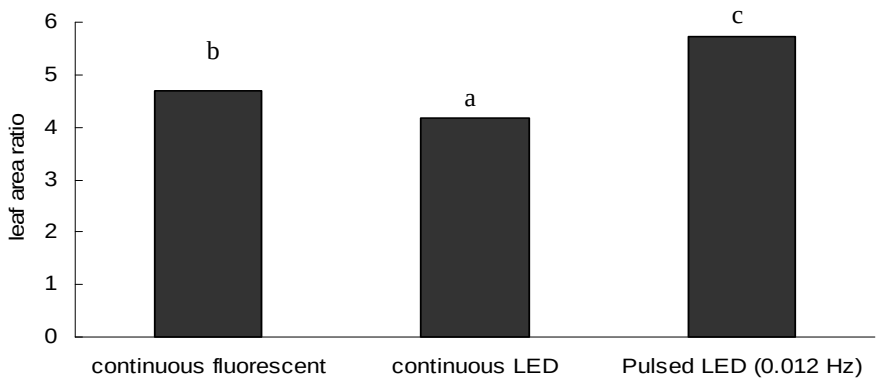

Figure 3.17. LAR (cm^2 mg^{-1}) of the various Lemna treatments. Different letters above the bars indicate significant (P<5%) differences as established by the LSD-test. LSD = 0.3543.

The average relative PSII efficiency over the different treatments was calculated and this showed that both LED treatments had a slightly lower efficiency as the fluorescent treatment, where the pulsed treatment is lower then the continuous treatment, except for the 100% actinic light. Furthermore when the actinic light intensity is increased, the efficiency decreases, not by much however (figure 3.18).

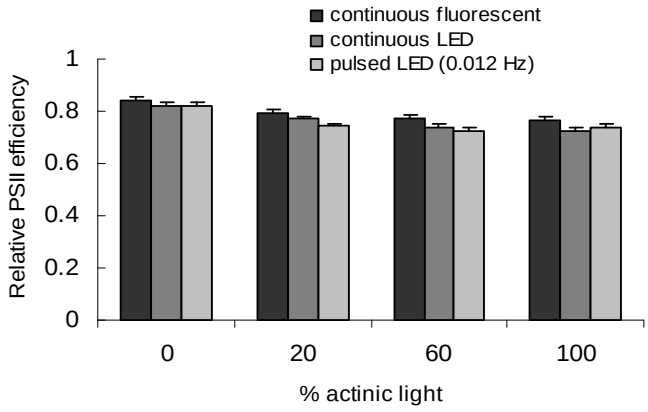

Figure 3.18. Average relative photosystem II efficiency over each treatment as established by chlorophyll fluorescence, error bars indicate standard deviations.

Experiment 6

For experiment 6 the frequency of the pulses was 120 Hz; so this is a factor ten higher then experiment 4 and a factor hundred higher then experiment 1. This was done to see if this would

result in an upper limit for pulsed light. The dry weight of the duckweed fronds on day 0 was calculated, and there was no significant difference (P=0.346) between the different treatments. The dry weight of the duckweed fronds at the end of the experiment was measured and there was no statistical difference (P=0.092) between the different treatments (table 3.10 and appendix E).

Table 3.10. Statistical analysis of the effect of the different treatments on the dry weight (mg) of the Lemna fronds and a comparison with the dry weight at the start of the experiment

Treatments	Dry weight (mg)	
	Day 0	Day 9
Continuous fluorescent	0.044[1] NS[2]	0.946 NS[2]
Continuous LED	0.047 NS	0.935 NS
Pulsed LED (120 Hz)	0.044 NS	0.819 NS

[1] Calculated as the area of the duckweeds present at day 0 * leaf area ratio of the continuous fluorescent treatment.
[2] NS indicates that there is no significant difference within a column (P<5%).

The relative area growth rate of the continuous fluorescent treatment was significantly higher (P<0.001) then the other two treatments (figure 3.19 and appendix E). The pulsed LED treatment had a significantly lower (P<0.001) leaf area ratio (LAR) then the other two treatments. The other two treatments also differed significantly, in that the continuous LED treatment had a lower LAR than the continuous fluorescent treatment (figure 3.20 and appendix E).

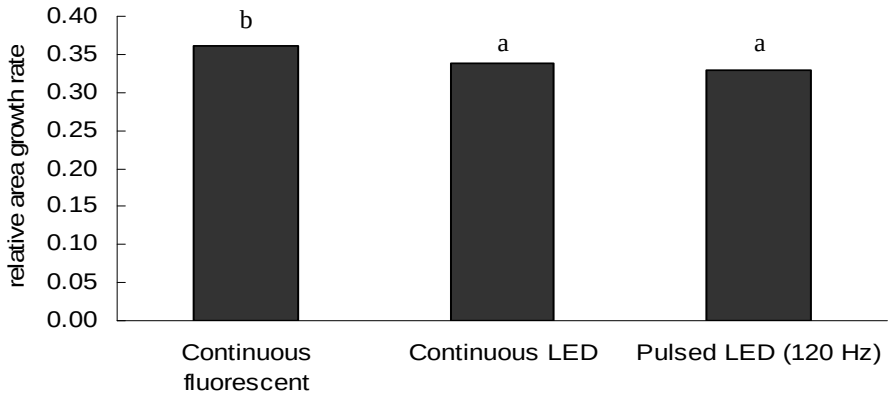

Figure 3.19. Relative area growth rate (cm^2 cm^{-2} day^{-1}) of the various Lemna treatments. Different letters above the bars indicate significant (P<5%) differences as established by the LSD-test. LSD = 0.00999.

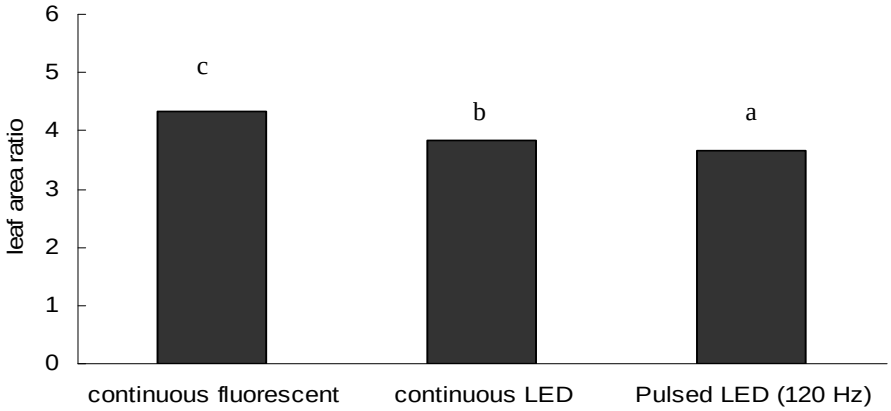

Figure 3.20. LAR (cm^2 mg^{-1}) of the various Lemna treatments. Different letters above the bars indicate significant (P<5%) differences as established by the LSD-test. LSD = 0.1714.

The average relative PSII efficiency over the different treatments was calculated and this showed that both LED treatments had a slightly higher efficiency as the fluorescent treatment in 0 and 20% actinic light, whereas it is the other way around for the 60 and 100% actinic light. The pulsed LED treatment is lower then the continuous LED, except for 100% actinic light. Furthermore when the actinic light intensity is increased, the efficiency decreases, not by much however (figure 3.21).

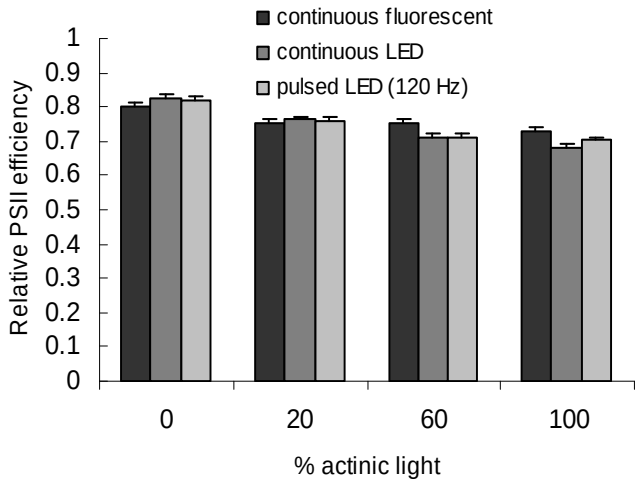

Figure 3.21. Average relative photosystem II efficiency over each treatment as established by chlorophyll fluorescence, error bars indicate standard deviations.

3.3 Comparison of the experiments

Finally the leaf area ratio and the relative area growth rate over all the experiments were compared, to see what the total effect was of the pulsing treatments. First a graph was made to check if the differences between the experiments for both LED treatments were similar to the differences between experiments for the fluorescent treatment (figure 3.22). It is clear that the growth rate of the pulsed treatment lies along the same line as that of the continuous treatment. But there is one outlying point at 0.012 Hz, where there is clearly a much lower growth rate. For the LAR it is obvious that that of the pulsed treatment is somewhat higher than that of the continuous treatment, where the most interesting point is at 0.012 Hz with a very high LAR and at 120 Hz with a quite low LAR.

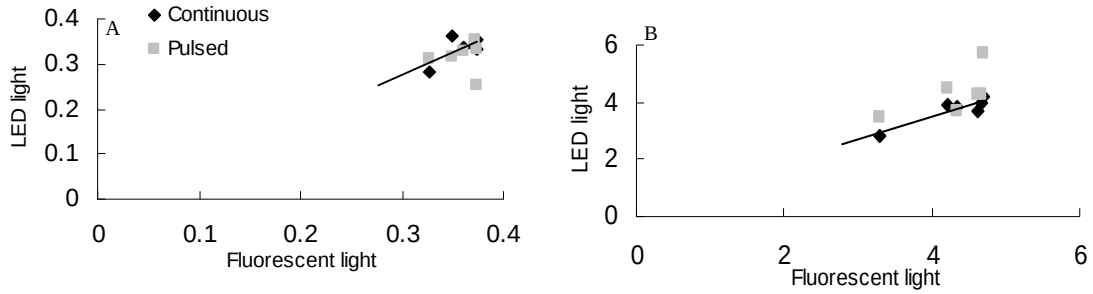

Figure 3.22. The relative area growth rate (cm^2 cm^{-2} day^{-1}) (A) and the leaf area ratio (cm^2 mg^{-1}) (B) of the LED treatments versus that of the fluorescent treatment. The trendline in both graphs is based on the continuous treatment.

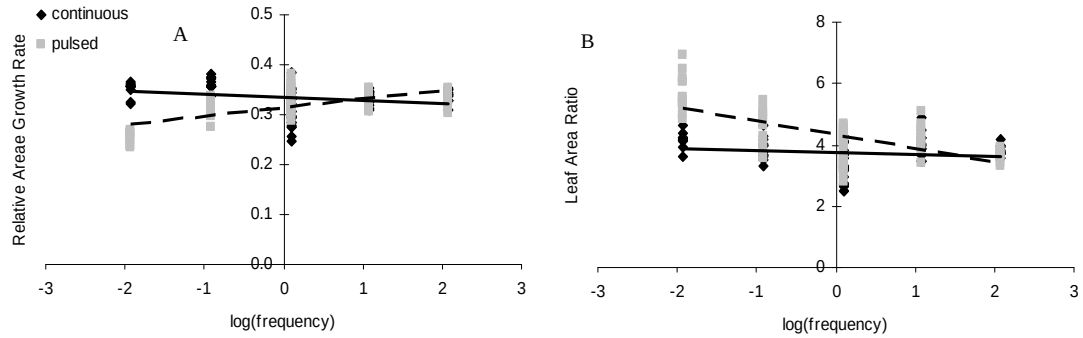

Figure 3.23. Relative area growth rate (cm^2 cm^{-2} day^{-1}) (A) and leaf area ratio (cm^2 mg^{-1})(B) of the two LED treatments, continuous and pulsed, versus the logarithm of the frequency of the pulses of the pulsed treatment. The uninterrupted line is the linear trend of the continuous treatment; the interrupted line is the linear trend for the pulsed treatment.

The effects seen in figure 3.22 where checked again by plotting the leaf area ratio and the relative area growth rate of both LED treatments versus the logarithm of the pulsing frequency (figure 3.23). To check whether there really was a difference between the two LED treatments, an one way ANOVA of the two linear trends was done and there was a statistical significant difference (P=0.050) between the two treatments for the leaf area ratio, but there was no statistical difference between the two treatments for the relative area growth rate (P=0.103) (appendix F).

Finally the comparison was made over the different times when the experiments were done, denoted as the frequency of the pulsed treatment, the results for the relative area growth rate are shown in figure 3.24 and the results for the leaf area ratio are shown in figure 3.25. According to this test there were significant differences (P<0.001) between the different experiments for the two control treatments, the continuous fluorescent treatment and the continuous LED treatment (appendix F). This means that there is a significant effect of the time when the experiment was done. The relative area growth rate will be discussed first, starting from the top to the bottom (figure 3.24). The relative area growth rate of the first 1.28 Hz experiment is the highest, together with the 0.012 Hz, 12 Hz and 120 Hz experiments, of which only the last one is not significantly higher then the 0.12 Hz experiment, which is significantly higher then the second 1.28 Hz experiment, which has the lowest relative area growth rate. In the continuous LED treatment the 0.012 and 0.12 Hz experiments had the highest relative area growth rate, followed by the first 1.28 Hz, 12 Hz and the 120 Hz experiments, which are significantly higher then the second 1.28 Hz experiment, which has the lowest relative area growth rate. In the pulsed LED treatment the first 1.28 Hz experiment had the highest relative area growth rate, followed by the 12 and 120 Hz experiments, followed by the 0.12 Hz and the second 1.28 Hz and the 0.012 Hz treatment had the lowest relative area growth rate (figure 3.24).

Next the leaf area ratio (LAR) will be discussed, also from the top till the bottom (figure 3.25). The LAR of the first 1.28 Hz experiment is the lowest, followed by the 0.12 Hz experiment, that is not significantly different from the second 1.28 Hz experiment and the 120 Hz experiment, that are both not significantly different from the 12 and 0.012 Hz experiment, however they are both significantly higher then the previously discussed experiments. In the continuous LED treatment the first 1.28 Hz experiment had the lowest LAR, followed by the second 1.28 Hz, 120 Hz and the 0.12 Hz experiments. Of these the 0.12 Hz and the 120 Hz treatments do not differ significantly from the 12 Hz, the 12 Hz treatment does not differ significantly from the 0.012 Hz, which has the highest LAR. In the pulsed LED treatment the first 1.28 Hz experiment and the 120 Hz experiment have the lowest LAR, followed by the 0.12 Hz, the second 1.28 Hz and the 0.12 Hz treatments, and all of these differed significantly from the 0.012 Hz experiment (figure 3.25).

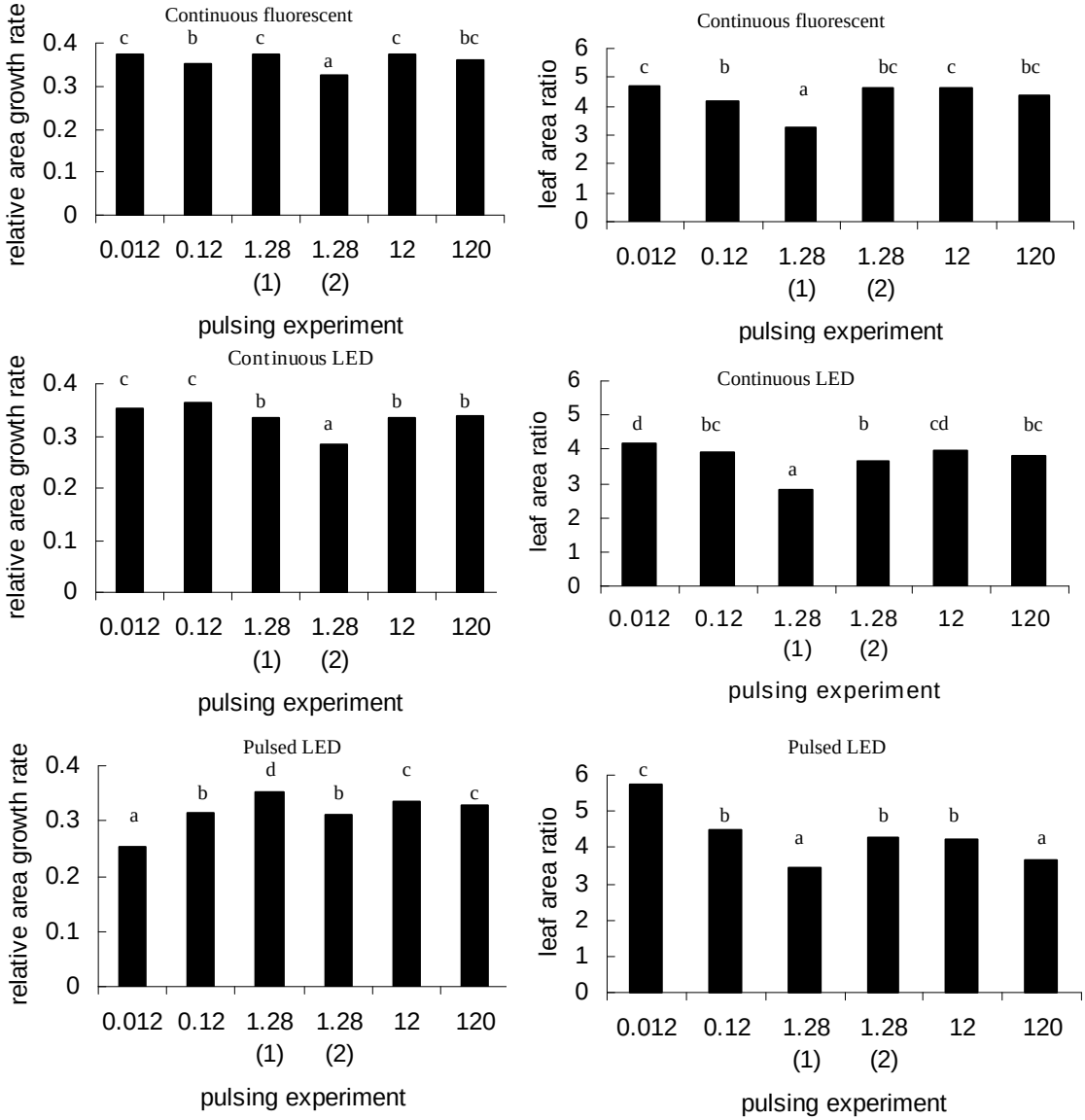

Figure 3.24. Comparison of the relative area growth rate (cm^2 cm^{-2} day^{-1}) of the different pulsing experiments over the three Lemna treatments. They are arranged from top to bottom, continuous fluorescent, continuous LED and pulsed LED, respectively. Different letters above bars indicate significant differences (P<5%) within a graph, as established by a LSD test, with LSD for CF= 0.01365; for CL= 0.01304; for PL = 0.01215.

Figure 3.25. Comparison of the leaf area ratio (cm^2 mg^{-1}) of the pulsing experiments over the three Lemna treatments. They are arranged from top to bottom, continuous fluorescent, continuous LED and pulsed LED, respectively. Different letters above bars indicate significant differences (P<5%) in a graph, as established by a LSD test, with LSD for CF= 0.4194; for CL= 0.2618; for PL = 0.3831.

4. DISCUSSION

4.1 Spirodela trial experiment

There was no significant difference of different nutrient solutions or photoperiods on the average area of the *Spirodela* fronds (table 3.1) and on the dry weight of the *Spirodela* fronds (table 3.2). This implies that a photoperiod of 16 hours and a nutrient treatment that was diluted by 50% (appendix A) could be used in the rest of this research. However, as can be seen in table 3.2, there is a very big difference between the different treatments; it is striking that this does not lead to a significant difference between the treatments. The most obvious reason for this is that there was a very big difference between the different samples within a treatment, which is probably the case. Furthermore the area measurements are not the most logical way of studying the growth, that is mainly caused by the dry weight measurement; this was done on every Monday, Wednesday and Friday, so that the average area was based on a decreasing amount of samples. The dry weight samples were taken in a logical way starting in one corner of the raster and working down the raster, however since not all of the samples were exactly the same at the start of the experiment, this lead to some strange results and very big variation. The most important outcome of this trial experiment was that the growth of *Spirodela* might not be the best model plant for this experiment, therefore as already stated in the material and methods section, the rest of the experiment was conducted with *Lemna minor* L.

The above discussion led to a set-up for the *Lemna* experiments with a photoperiod of 16 hours, a 50% dilution of the standard tomato nutrient solution and most importantly a sample method that made sure that the area measurements were based on as much samples as possible. So the dry weight measurement was done only at the end of each experimental period and the dry weight at the beginning of each experimental period was calculated.

Another important outcome of this trial experiment was that the duckweed fronds were able to grow quite normally under the red LED array, since there was no statistical difference of the light source on the average area of the *Spirodela* fronds (table 3.3) and on the dry weight of the fronds (table 3.4). The same explanations for the fact that there is no statistical difference as in the previous part of this discussion can be given. This is again mainly caused by the taking out of the fronds during the growth period, to use them in a dry weight measurement. Whereas the lack of a significant difference in the dry weight measurement could be caused by the large variation in different samples within a treatment as already discussed previously. However, it has been shown that plants grown under red light alone leads to an excessive increase in hypocotyl elongation,

furthermore it increases the length of leaves, but probably solely by increasing the length of the petiole (Hoenecke et al., 1992). This does not lead to any problems with the duckweed species, because they do not contain any hypocotyl tissue and the leaves, or leaf like bodies, do not have any petioles to speak of (Cross, 2002).

4.2 Pulsing experiments

The wavelength of the light sources used in this research (figure 3.1) show that the red LEDs emit their radiation in an area where leaves absorb this radiation very efficiently (figure 1.4), whereas a lot of the radiation emitted by the fluorescent tubes falls into zones where leaves are not very efficient in their absorption. Therefore these LEDs would be more efficient sources of assimilation light for the growth of plants, then the fluorescent tubes, but this is only the case when the development and morphology of the plants is not changed by the monochromatic light source. However multiple reports indicate that the addition of some blue light is essential for the healthy development of plants (Yorio et al., 2001; Okamoto et al., 1996).

The 'sinusoidal' growth patterns of the Lemna fronds (figure 3.2) is probably caused because the plants accumulate starch one day, and only invest sugars on the outgrowth of existing fronds, and on the following day use some of the accumulated starch together with the newly acquired sugars to create new fronds, however this explanation is not grounded with experimental data.

The agreement of the calculated area growth rate with the measured area of the duckweeds was as expected, since plants normally grow in an exponential growth pattern. Sometimes some deviations from this pattern occurred, but that was probably caused by duckweed fronds growing over each other, so that not all of the area present can be measured. Furthermore, the overgrowth also causes self-shading, which leads to growth reduction of the fronds (Szabó et al., 2003).

Red light effects

In general the relative area growth rate of the continuous LED treatment is lower than that of the continuous fluorescent treatment (for example figure 3.7), however some exceptions occur like in experiment 3 where the continuous LED treatment has a slightly higher relative area growth rate, but not significantly so (figure 3.10). So it seems like that even for *Lemna* it is not possible to grow normally under monochromatic red light alone. This conclusion seems justified from the results of the PSII efficiency, since in almost all experiments and over all actinic light percentages, except for experiment 5 at 0 and 20% actinic light (figure 3.21), the continuous LED grown plants have a lower PSII efficiency than the continuous fluorescent grown plants. This seems to be in agreement with previous research where a lower photosynthesis efficiency and a lower stomatal conductance was also reported for plants grown under red LEDs, compared to a white xenon arc lamp. However, they reported that the photosynthesis efficiency was higher for the LEDs under low light intensities (<300 $\mu mol\ m^{-2}\ s^{-1}$), which were also used in this experiment

(Tennessen et al., 1994). But this lower PSII efficiency in this experiment is probably caused because of the measurement method. The measurement lights of the Fluorcam are red-orange LEDs that are a little different from the LEDs under which the plants where grown. The same off course holds for the plants under the fluorescent light, this is also different from the light of the Fluorcam, but a different response probably occurs. Overall the differences in PSII efficiency found in this experiment are so low, however, that it is not certain that this is caused by the red light and could depend on the measurement method. Since the applied actinic light intensities where very low (<30 $\mu mol\ m^{-2}\ s^{-1}$), there is not the clear effect one would expect of increasing the light intensity, this is caused by the way the fluorescence pictures were taken, and it applies for all of the PSII efficiency data discussed in this report. The entire aquarium was placed under the imager for each picture; therefore it had to be some way below the camera, and thus below the actinic light sources. There is hardly ever an effect of the use of red LEDs on the dry weight of the *Lemna* fronds (table 3.5 through 3.10), except for experiment 4, where the continuous LED grown plants had a lower dry weight then the continuous fluorescent grown plants (table 3.8). This is probably caused by the fact that in the calculated dry weight on day 0 there also was a significant difference and that the continuous LED grown plants where not able to eliminate this difference over the growth period.

From the area growth rate and the dry weight of the fronds, the next results appear to be logical, in that the leaf area ratio is usually lower for the LED grown plants compared to the fluorescent grown plants (figures 3.5, 3.8, 3.14, 3.17 and 3.20), except for experiment 3 where there is no difference (figure 3.11), but there, there was also no difference in the area growth rate. This would mean that the development of *Lemna* under red LEDs is altered compared to under white fluorescent tubes. So under the red LEDs the fronds get thicker and longer roots develop, which is something that can also be seen in sun leaves, compared to shade leaves, which stretch further and are thus slimmer (Raven et al., 1999). This effect of sun and shade leaves is linked to the red/far red ratio of the light a plant receives, which is far higher for the plants grown under the red LEDs, so they can be seen as sun leaves (figure 3.1).

When looking at the graph of the LED light versus the fluorescent light (figure 3.22), then it is clear that the datapoints of the LEDs both for the relative area growth rate and for the LAR there is a clear line. So the variation that is present in both control treatments appears to have the same causes. One of these causes could be that the nutrient solution used in the experiment was not of a consistent nature, the EC of this solution was significantly different for each experiment (data not shown).

Pulsed light effects

As can be seen from figure 3.23 the LAR decreases from the lowest pulsing frequency till the highest frequency and this trend is significantly different from the trend in the continuous LED treatment, which is very close to a straight line. For the relative area growth rate this difference does not occur, so the trend visible in that part of the graph seems a bit unrealistic. However, there will be some differences in this variable over the different experiments. At a pulsing frequency of 1.28 Hz, in both repetitions the pulsed LED treatment had a higher relative area growth rate (figures 3.4 and 3.7), an equal dry weight (tables 3.5 and 3.6), and thus a higher leaf area ratio (figures 3.5 and 3.8) then the continuous LED treatment. The results from the fluorescence measurements showed that there hardly was a difference in PSII efficiency (figures 3.6 and 3.9), and if there was a difference the pulsed LED grown plants had a lower efficiency. It is obvious from these results that the plants adapt their photosystems, and thus their entire photosynthesis to the pulsed light. The Lemna fronds under the pulsed light seem to operate less efficient, since they need a higher amount of photosynthetic tissue, the area of the fronds, to be able to produce the same amount of dry weight. From the experiments of Chua and Dickson (1964), who also used pulsed lighting for the growth of duckweeds, it seems that at a flash time of approximately 0.4 seconds, as in this experiment, the gain in dry weight over 72 hours is about 300% higher than for a light period of 12 hours, this is different from what is found here. They suggest that it is caused by the flash frequency and the length of the dark period. So that it should lie in the dark period, but then it is strange that other results are found here. However, they look at treatments with similar light intensities, and not with similar daily light sums. Furthermore, they did not measure any area growth, thus the comparison can not be taken any further. Poni and Intrieri (2001) showed that at a frequency of 1.33 Hz, the photosynthesis efficiency of vine leaves is approximately 75% of the photosynthesis in full sunlight; however they did not receive the same photon flux density, so the comparison is not really valid. Therefore it is quite well possible that the photosynthesis rate of the plants at 1.28 Hz is the same as for those under continuous irradiation. The effect of the daily light integral that is the only difference between the two 1.28 Hz treatments will be discussed in the next paragraph.

At a pulsing frequency of 0.12 Hz, so a pulse duration of 4 seconds, followed by an equal dark period, the relative PSII efficiency is much lower for the pulsed LED treatment than for the continuous LED treatment (figure 3.12). This results in a lower dry weight of the pulsed LED treatment (table 3.7), and because of the shortage of assimilates a lower area growth rate (figure 3.10). However, the plants try to adapt to this environment by using as much of the limited

amount of assimilates available to produce fronds, to intercept as much radiation as possible to increase the assimilate production, as can be seen from the higher LAR (figure 3.11). From the review of published data by Sager and Giger (1980), it is shown that for almost all higher plant species at a duty ratio of 1 and a pulse duration of ± 4 seconds, the plant growth is reduced. The same holds for the data of Chua and Dickson (1964), however in both cases there are no data points at this frequency, it is an interpolation from the graphs.

At a pulsing frequency of 12 Hz, so a pulse duration of 0.4 seconds, followed by an equal dark period, the relative PSII efficiency is slightly lower than that for continuous LED light (figure 3.15), resulting in a lower dry weight for the pulsed treatment (table 3.8). However, both the relative area growth rate (figure 3.13) and the LAR (figure 3.14) are not significantly different. This indicates that the plants under the pulsed light are not triggered to increase there leaf area to increase the assimilate production. This is in clear disagreement with data published by Chua and Dickson (1964), as well as by others mentioned in their article, which show a definite increase in the dry weight production at a duration below 1 second compared to at a photoperiod of 12 hours. But they compare equal light intensities, and not equal light sums, like in this experiment, furthermore their data were unsuitable for the analysis of Sager and Giger (1980), so the relative efficiency of intermittent versus continuous light is not known. Since the report of Chua and Dickson (1964) is one of the few known experiments on the application of pulsed light on *Lemna* species however, it remains interesting to compare this experiment with their data.

At a frequency of 0.012 Hz, so a pulse duration of 40 seconds, followed by an equal dark period, the relative PSII efficiency of the fronds under pulsed light is slightly lower, but more or less equal to that of the continuous LED treatment (figure 3.18). However, the dry weight of the plants under the pulsed light is lower (table 3.9), so this is a result of another limitation in the path of photosynthesis to plant growth. The relative area growth of the fronds under the pulsed light is also decreased (figure 3.16), probably because of the limited amount of assimilates available to produce new tissue. All in all it seems like the area growth is much less reduced then the dry weight, since the LAR is much higher for the pulsed light grown plants (figure 3.17), it even exceeds the LAR of the plants grown under the fluorescent light, and thus overcomes the usual effects of red light. Chua and Dickson (1964) show the same decrease in dry weight, around a pulse duration of one minute, they even say that this treatment results in the lowest dry weight. This seems to agree well with these data, it is strange however that this does not seem to be an effect on the photosynthesis efficiency, but that it has another cause. It is not sure what this cause could be, however. It should be kept in mind that with this experiment the frequency had to be checked on a daily basis, since it was at the absolute minimum of the capability of the oscillator.

At a pulsing frequency of 120 Hz, so a pulse duration of 0.004 seconds, followed by an equal dark period, the relative PSII efficiency of the pulsed treatment was equal to or even slightly higher than that of the continuous LED treatments (figure 3.21). As a result of this similar efficiency, the dry weight of the plants under the pulsed light was also equal to those under the continuous light (table 3.10), the area growth rate of the plants under the pulsed light was also equal to that under the continuous LED light (figure 3.19). However, when we look at the LAR, the pulsed LED plants have a lower LAR then the continuous LED plants (figure 3.20). The plants react as though they have no need to invest in tissue to harvest more light, and can in stead of that invest their assimilates in root tissue and thicker fronds. It could be the case that this is an effect of the nutrient solution in which the *Lemna* where placed, since the EC was measured, and did not differ between the treatments within an experiment, but that does not say anything about the availability of single nutrients. Furthermore the uptake characteristics of fronds and roots of *Lemna* differs (Cedergreen and Madsen, 2002), so when the environment changes than the *Lemna* will adapt by producing different tissue types. However it is strange that this happens since it is something that goes straight against the response in other experiments.

4.3 Comparison between the experiments

Over time, so between the experiments, differences occurred both in the relative area growth rate and in the LAR of the continuous fluorescent treatment (figures 3.24 and 3.25), the control treatment. These results could have different causes, a few of these are; variations in the starting material of the *Lemna*. Variations in the climatic conditions inside the climate chamber, mainly caused by the amount of people visiting it and the time they spent inside, this if nothing else causes a higher CO_2 concentration inside the climate chamber. In the light intensity of the fluorescent tubes, this decreased somewhat over time (table 2.4). Furthermore there can always be differences in the amount of algae present in the air, nutrient solution, etc. The nutrient solution was also varying over time, although it is taken out of a stock solution, which is made in large quantities, four separate batches were taken out, to be used in these experiments. The EC of the diluted nutrient solution for each experiment differed between each experiment. So this could be a cause of the variation. Which of these, or all at the same time, is/are the causes of the differences in the LAR and relative area growth rate over time is not known. The variation in the dry weight is not discussed, since that is too much depending on the size of the fronds taken out at the beginning of the experiment, and that this varied very much between and within experiments.

For the continuous LED treatment, that is the control treatment mostly correlated with the pulsed LED treatment, because of the same light quality and light source, there also was a difference between the different experiments for the LAR and relative area growth rate (figure 3.24 and 3.25). What is more interesting though is that the pattern of differences in the continuous LED treatment is the same as the pattern in the continuous fluorescent treatment (figure 3.22). So the differences between the experiments leading to the variations in relative area growth rate and LAR are the same for both treatments, this would mean that they are probably also the same for the pulsed treatment, and since it is shown that for the LAR the trend in both LED treatments is different, there will be a real difference in these data (figure 3.23). Small deviations in the variation of the LED treatment compared to the fluorescent treatment could be caused by a variation in the light intensity that decreased over time in all treatments. This decrease is different for the continuous LED treatment compared to the fluorescent treatment. The fronds that were put into the experiment at the start also showed a lot of variation, although when comparing the dry weight on day 0 there usually was no difference between the continuous LED treatment and the continuous fluorescent treatment, it could cause some differences when comparing within one treatment.

For the pulsed LED treatment, as compared to the continuous LED treatment, first the most striking differences will be discussed. At 120 Hz the LAR is very much lower, than is seen in the other experiments. Here probably the physiology of the plants is adapted to the pulsed light in such a way that it has less need to produce a high amount of photosynthetic tissue. So the adaptation that usually occurs in the red light is not reversed in this particular experiment, like it usually occurs when pulsed light is used. For the relative area growth rate the very low value at 0.012 Hz is striking. This is probably caused by the lower photosynthesis, which made it hard to grow fast for the plants in this experiment. When comparing the LAR with the relative area growth rate between the pulsed LED and the continuous LED treatment, there are some strange results.

At 0.012 and 0.12 Hz the lower relative area growth rate was combined with a higher LAR, but at 1.28 Hz the higher relative area growth rate was combined with a higher LAR. The cause of this is probably to be found in the stretching of the fronds to capture as much of the radiation as possible. This happens at all of these frequencies, however because there are more assimilates available in the 1.28 Hz experiments, they are also capable of growing faster and thus have a higher relative area growth rate, whereas at the 0.12 and 0.012 Hz treatments there are not so much assimilates available. So they try to get a large amount of photosynthetically active tissue, like shade leaves do. Also like commonly seen for shade leaves the leaf specific weight, expressed as the leaf area ratio, its inverse, is lower (Poni and Intrieri, 2001). But because of the limited assimilate availability this does not lead to a higher area growth. Furthermore the dark periods after each pulse in the 1.28 Hz experiments still had a light intensity of approximately 28 $\mu mol\ m^{-2}\ s^{-1}$, because the pulse stopper was not yet completed at the moment these experiments were run. It is not really clear what the effects of such dark pulses will be on the plants, to conclude anything about that an experiment should be done where the dark pulse would be completely dark and compare it.

At 12 and 120 Hz the relative area growth rate of the pulsed LED grown plants is equal to that of the continuous LED treatment. For the 12 Hz experiment the LAR is also equal, but for the 120 Hz experiment the LAR is lower. This would indicate that at 120 Hz the pulsed LED treatment triggers the fronds to act more like a sun leaf than the continuous LED treatment, resulting in a lower stretching of the fronds. However, because there are enough assimilates available, the relative area growth rate is the same for these experiments.

Finally the comparison between the two 1.28 Hz experiments will be discussed, so a comparison where one experiment had a slightly higher DLI than the other. The higher DLI leads to a higher relative area growth rate, but not too a difference in the LAR. This means that the

physiology of the plants is not altered by having a higher DLI, but that only the growth is altered. However according to experiments done by Aziz and Kochi (1999) with *Spirodela polyrhiza* and *S. punctata*, not only does lowering the light intensity and thus the DLI lead to a lower fresh weight, but also to a decreased fresh weight per frond. This would mean a lower leaf weight ratio and thus a higher leaf area ratio. Also in a study mentioned by them, in *Lemna* an increased surface to weight ratio was observed with a rise in light intensity (Ashby and Oxley, 1935). However, in both cases the increases in light intensity are of a very high order, double or even more, whereas in this experiment it was only a very low increase in light intensity, of ± 7.5% (table 2.3). In the study of Aziz and Kochi (1999) the amount of chlorophylls per sample also decreases with decreasing light intensity, this is also not noted in this study, as expressed by the greenness of the fronds. Again this could be caused by the very low decrease in light intensity, and by the method of analysis of these data.

4.4 Recommendations for further research

Pulsed lighting

The equipment and the experience of applying pulsed light with LEDs is now available, the causes of the differences in response to pulsed light at different frequencies have not been found out completely though. Therefore it would be very interesting to investigate different aspects of the photosynthesis as only PSII efficiency and growth. For example an interesting thing would be to see how the stomata react to the pulsed light and more importantly how the stomata react in combination with the photosynthesis efficiency and the chlorophyll content of the fronds is also very interesting. Furthermore the effect of the pulsed light around a frequency of approximately one Hertz deserves a lot of attention. It has been shown here that a distinctly different response occurs there. However, it is interesting to see whether this effect has been caused by the fact that the 'off'-signal still had a light intensity of 28 $\mu mol\ m^{-2}\ s^{-1}$, or by the frequency of the pulsed light. Last but not least pulses at a microsecond scale would also be very interesting for future research.

Working with Lemna

When working with *Lemna* it is of major importance to keep the level of algae in the nutrient solution as low as possible, this means that everything has to be disinfected. It would also be nice if there is a filter installed in the climate chamber to take as much algae out of the air as is possible. Furthermore it could be a good idea to wear gloves or to disinfect ones hands when working with *Lemna*.

Next to this it is nice to always start out every experiment with the same genetic batch of *Lemna*; therefore they should be grown out of one mother frond that is disinfected. This was not done in this experiment, and could be seen as a small error. However, since they were taken out of the same part of the same ditch, one would expect a low amount of genetic variability, but it is always possible that there is some variability.

5. CONCLUSIONS

- LEDs are good light sources for studying the effects of pulsed light.
- *Lemna minor*, although it is an aquatic plant, is a good model plant to study the effects of different environmental factors on photosynthesis of higher plants.
- It is possible to grow plants under pulsed light.
- Pulsed light has differing effects when the frequency of the pulses is altered, but there is always an effect of the pulsing.
- The photosynthesis efficiency of the plants under pulsed light can only approach, but never exceed the photosynthesis efficiency of the plants under continuous light with the same daily light integral.
- The dry weight accumulation under pulsed light only approaches the dry weight accumulation under continuous light, but never exceeds this.
- The photosystem II efficiency alone is not enough to explain all of the effects encountered in these experiments. The effects of pulsed light are also caused by different processes, probably also related to the dark reaction of photosynthesis.
- The exact causes of the differing effects at different frequencies has still to be found out.
- If pulsed LED light is used to grow plants, then the frequency of this pulsing should always remain above 1 hertz, to maintain a more or less normal plant growth.
- If the frequency of the pulses is increased from 0.012 Hz till 120 Hz, the leaf area ratio decreases. The relative area growth rate appears to remain almost equal over the same range, or even increases slightly.

It is possible to grow plants under pulsed red LEDs, however some morphological features of the plants change, furthermore most of the time it is not beneficial to grow plants under the pulsed light. A lot of interesting things are going on in the plants, so this remains an area of considerable interest for future research.

6. LED ARRAYS

6.1 Introduction

To investigate the effects of pulsed light provided by LEDs it was necessary to build LED arrays which were capable of providing enough light output over a sufficiently large area. Since some arrays where already available from previous experiments (Lavrijsen, 2003), they were used as a basis for the calculations on how the new LED arrays should look, and how they should be build. Especially since the new arrays had to be capable of working with the same current supply as was used for the earlier arrays (Lavrijsen, 2003). However there also had to be some new design features for the new arrays, for example a break-out box was made to make sure that the arrays could not be short-circuited, and which was also able to protect the array from a too high current. Furthermore a box which could turn off the power completely at night was also made. This box contained a relay which could switch the current flow off when it had no power and switch the flow on when it was powered. And for the pulsed treatment a special box was made that turned the current completely off, since there always was some leakage current when the signal should be off (Lavrijsen, 2003).

6.2 materials and methods

Input for Matlab scripts

Single LEDs were mounted on a copper ‘pole’ that served as a heatsink. Eight of these ‘poles’ were made, they had a diameter of twelve mm and a length of ten cm. The upper part of the ‘poles’ was made absolutely flat. On this upper part single LEDs were mounted with ‘seconde lijm’ (figure 6.1). After the LEDs had been attached to the poles they were wired. The LEDs were hung on a movable frame, which was at five cm above the quantum sensor, this frame made it possible to move the LEDs ten cm relative to the quantum sensor (figure 6.2). The LEDs were run at a current of 20 mA except for the amber coloured LED that had to be run at 30 mA in order to see the output of the quantum sensor (figure 6.3). This current was applied by a Voltcraft (DPS-4005 PFC) variable DC current source.

Figure 6.1. Design of the LEDs mounted on the poles, in order to test their light intensity.

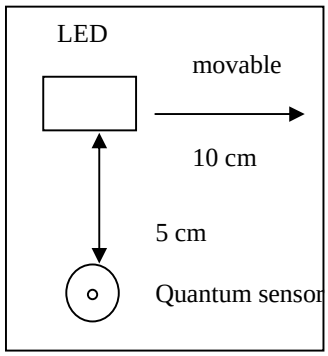

Figure 6.2. Drawing of the set-up of the light intensity measurement. Modified from Lavrijsen, 2003.

From these light intensity measurements a polynomial was estimated to model what the light intensity will be at each distance from the LED (table 6.1). These polynomials are the main input for the calculations in the Matlab program, which is described in the next section. Five of the main colours of LEDs were tested in such a way, however the remainder of this chapter will only focus on the red one, since this was the only one from which an array was actually build.

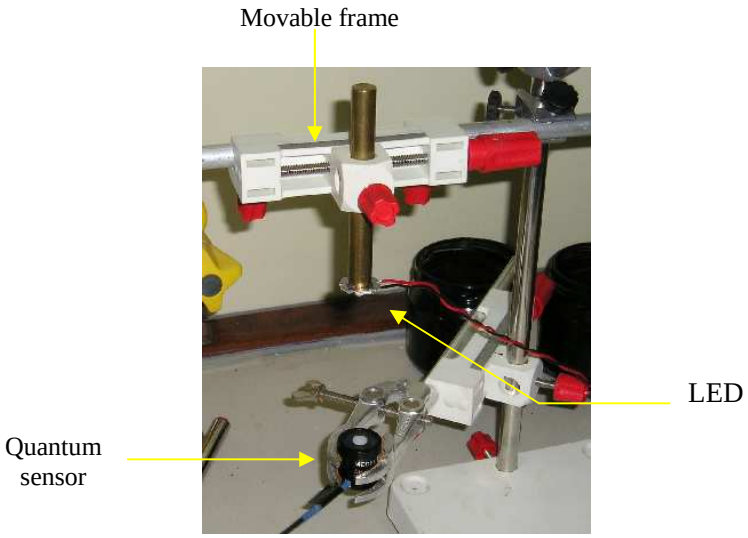

Figure 6.3. Design used for testing the light intensity of the LEDs.

Table 6.1. Polynomials estimating light intensity at distance x for each LED

Amber	$y = -0.001*x^2 + 0.0041*x + 2.7298$
Blue	$y = -0.0012*x^2 + 0.0086*x + 4.5825$
Cyan	$y = -0.0005*x^2 + 0.0087*x + 2.1815$
Green	$y = -0.0006*x^2 + 0.0005*x + 2.5285$
Red	$y = 0.0002*x^2 - 0.0827*x + 6.519$
Red-Orange	$y = -0.0021*x^2 + 0.042*x + 5.441$

Matlab scripts

In order to calculate the variability in the LED-arrays which where build, Matlab (Matlab release 11, version 5.3, 1999, The Mathworks inc.) script files have been written (Harbinson, 2004). Multiple scripts are needed to calculate the variability; they are included in appendix G and will be discussed here to show how they were used and to make sure that they can be used easily by other people when they have read through this text. In this paragraph all variables are written in the same way as in the scripts. The scripts are:

- Make_list
- Index_vec
- Red (red can be substituted by any of the colours made, by changing the polynomial used)
- Diversity

The file red starts by asking you how big the array should be, in order to calculate that it asks for the size of one side of the square. This is done because the only shape of array possible in this program is a square shape. The program continues by asking for the LED locations, they must be given as an array like this [a b; c d; etc]. From the array of LED locations the program calculates the amount of LEDs that are inserted and gives this value as loop_number. Subsequently it uses the make_list program to make a list of grid cells. Which is calculated as 1 till the grid_size squared and transposed in order to create a column. The variable created is grid_cells. Thereafter space is allocated for the irradiation output, this is done by making an array of zeros with the width of loop_number and a length of grid_size squared.

The following step is the loop that calculates the distance between each cell. The loop starts by creating location, which is repeated as many times as there are LEDs present. And for each of these repeats it calculates the distance by using the file index_vec. This file needs grid_cells, grid_size and each single led location, defined by in which loop it is, which is defined by location, as inputs. From these inputs it calculates the distance that each single position has from each LED. However the distance which has now been calculated is not the real distance, because the program calculates the distance between each cell, the real distance is given by multiplying the distance calculated by 7, because the LED diameter is 7 mm. Based on the distance the light intensity is calculated, for this calculation a polynomial function was created by measuring the light intensity of each colour LED at increasing distance and using these data for making the polynomial. The light intensity is checked for negative values, because negative light intensities are impossible. The last item of the loop creates irradiation arrays for each LED. These irradiation

arrays are the same as the light intensities, except that the negative values are substituted by zeroes. Then the loop is ended.

Then x is defined as the sum of all the irradiation arrays for each position. These x values are then reshaped, which means that the values remain exactly the same, only their position changes. The variable x is a list of light intensity values; however the initial array was a square. So the variable y is created in which x is reshaped into the initial square. The amount of LEDs present is shown in the output by the command loop_number.

Subsequently a figure is created which consists of two subplots. The first subplot contains a colourmap of the light intensities. The second subplot shows the locations of all LEDs present. At last variables are calculated in the file diversity; with these variables it is possible to show the variation in light intensity over the array. It uses the light intensity array and the grid size as inputs. From these inputs it calculates the variables z and number. z is a column of percentages of the maximum light intensity, and number is a line of numbers ranging from 1 till the grid_size, which is needed for the plotting of z. This plot can be made by inserting the command 'plot(number, z(51,number))' the 51 which is stated here could be replaced by every number one wants to plot, this number stands for the position on the x-axes from which one wishes to plot.

These scripts could be used for the calculation of other light distributions of for example greenhouse lighting. However some lines then have to be altered for the program to work properly, these are lines 17 and 19 in file red (these lines have been marked in the appendix (G) with an asterisk (*)). Line 17 has to be altered, so that the real distance is calculated, this could be different when another light source is used. Line 19 has to be altered so that the real light intensities are calculated, for this purpose other polynomial functions should be established, based upon light measurements taken from the light source. And of course some titles could be changed, in accordance to the light source used.

A problem that occurs in all colour files is that they cannot calculate the light intensity if there is only one light source present. In order to overcome this, on line 16 after led_locations (location,:) needs to be removed (marked with a plus sign (+)). And on line 27 x= sum(irr_array,2) needs to become: x= irr_array(:,2) (marked with a minus sign (-)). Then it calculates everything correctly if only one light source is present.

Light intensity

An experiment was done to check how many LEDs are needed to achieve the light intensity needed by the plants, in order to make the calculation possible, a light intensity of 200 µmol m^{-2} s^{-}

[1] was chosen. This calculation was done in Microsoft excel (office 97 edition, 1997). The LED arrays build by Ad Lavrijsen (Lavrijsen, 2003) were tested for their light intensity output at 1.8 A (measured with a Voltcraft® GDM707 graphical display meter), this means 162 mA per LED (24 LEDs per array). All of the available arrays were used for this calculation, so that there could be a comparison between the different colours and their output. This output was then input for the excel calculations. Which are shown and explained in table 6.2. The amount of LEDs shown here might be on the optimistic side, probably more are needed. The maximum current for these LEDs is 350mA which is used for this calculation; however it is not wise to drive the LEDs at this maximum, since this will diminish both the lighting hours and the efficiency of these LEDs.

Table 6.2. Calculation of the amount of LEDs needed for an average light intensity of 200 µmol m^{-2} s^{-1}.

colour	Light intensity		Light intensity/LED[2]	# LEDs needed[3]
	At 162 mA	At 350 mA[1]		
Amber	71.7	154.2	6.4	31
Yellow	53.9	115.9	4.8	41
Green	59.5	127.9	5.3	38
Red	205.8	442.5	18.4	11
Blue	120.8	259.7	10.8	18

[1] Calculated as the maximum forward current divided by the current used for measurement multiplied by the measured value of the light intensity
[2] Calculated as the light intensity at the maximum divided by the amount of LEDs present
[3] Calculated as the light intensity desired divided by the light intensity per LED, rounded off to an integer value.

Figure 6.4. LED array covered with aluminium foil, to test the light intensity.

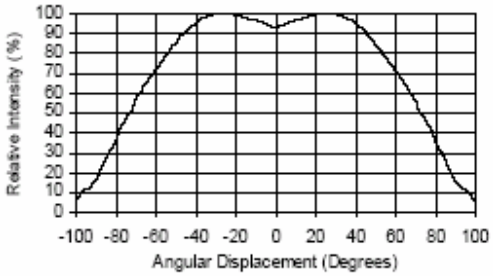

Figure 6.5 Typical Representative Spatial lambertian Radiation Pattern for Luxeon Star Red, Red-Orange and Amber (luxeon datasheet DS23, 2003).

A second experiment was run where the arrays were covered with aluminium foil (figure 6.4). This was done because aluminium foil reflects nearly all of the light, so nearly all of the light can be captured with the quantum sensor. This resulted in a much higher light intensity per array, which clearly indicates that much of the light that is emitted by the LED arrays is emitted

sideways and not directly down. This leads to the conclusion that when these arrays are used it would be wise to cover the sides of the area that has to be emitted; so that all of the light reaches the place it is intended to reach. This particular feature is caused by the type of LEDs used and their design (figure 6.5). However all of the data given here are for an area as big as the original arrays that were tested. The new arrays had a much larger area, to light a greater area.

Table 6.3. Calculation of the amount of LEDs needed for an average light intensity of 200 µmol m^{-2} s^{-1}.

Colour	Light intensity		Light	# LEDs needed[2]
	At 54 mA[1]	At 350 mA[2]	intensity/LED[2]	
Red	345	2236	93	3
Blue	335	2171	90	3
Cyan	115	745	31	7
Green	112	725	30	7

[1] Average values are taken over 3 measurements and rounded off to 0 decimals.
[2] All calculations performed as in table 6.2.

After some careful consideration of the above mentioned results the choice was made to keep on the safe side and also be able to use low duty cycles, the number of LEDs per array was set at 24 for the normal array, and 48 for the array that would be used for the pulsing. These were divided over an area of 300 cm^2 on a heatsink with an area of 500 cm^2, so the actual lit area was 500 cm^2.

LED characteristics

An important aspect of LEDs is that they are current driven, and thus not voltage driven. Their luminous output rises with rising forward current, under while just slightly increasing in the Forward Voltage (Datasheet Luxeon DS23, 2003).
Important for the LEDs used is the maximum average forward current (I_{AF}) they can take and the voltage across them when operating at this current (V_{TYP}) (table 6.4). To ensure right operation and to protect the LED respectively, the voltage across the LED must be higher than V_{MIN} and may not exceed V_{MAX}.

Table 6.4 Electrical LED characteristics. (Modified from Datasheet Luxeon DS23, 2003)

I_{AF} (mA)	V_{MIN} (V)	V_{TYP} (V)	V_{MAX} (V)
350	2.31	2.95	3.51

When LEDs are run at high power, they tend to heat up, this leads to a higher junction temperature within the LEDs, when the junction temperature gets too high, the luminous output of the LEDs changes. Therefore when in operation the LEDs have to be cooled, this can be

achieved by mounting them on a heatsink and placing a PC cooling fan on the back of the heatsink. But the leads and the slug (which is not electrically neutral) must be isolated from the heatsink to prevent short-circuiting. This problem was overcome by spraying a layer of plastic on the heatsink and bending the leads of the LEDs away from the surface of the heatsink (figure 6.6).

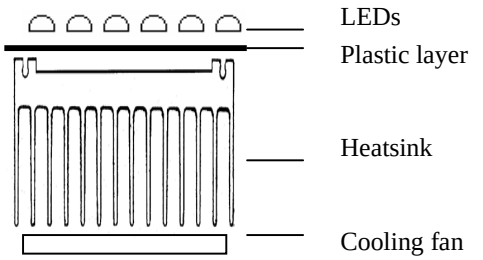

Figure 6.6 Construction LED-array. (Modified from Lavrijsen, 2003).

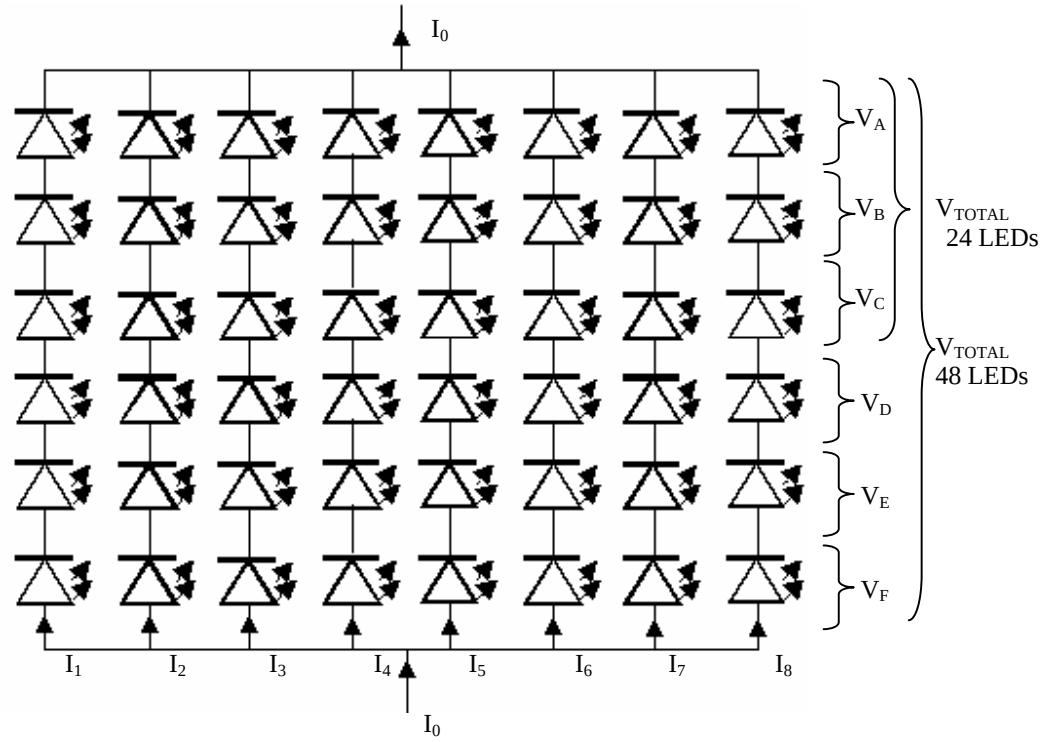

Figure 6.7. Circuit diagram LED-array (24 or 48 LEDs). (Modified from Lavrijssen, 2003).

The slugs of the LEDs are mounted on the heatsink with thermo conductive glue to provide adequate heat exchange. After that the leads are soldered in series with wire between them. One array, the one made to be pulsed, was constructed with 48 LEDs, resulting in six LEDs in series

and eight series wired in parallel (figure 6.7). The other array was constructed with 24 LEDs, resulting in three LEDs in series and eight series wired in parallel. With figure 6.7 and the values from table 6.4, the total I_{AF}, V_{MIN}, V_{TYP} and V_{MAX} for the LED-arrays are calculated (table 6.5), using the following equations:

$$\text{Total } I_{AF} = \text{sum } \{I_1 \ldots I_8\} = 8 * I_{AF} \quad \text{(eq 6.1)}$$

$$\text{Total } V_{MIN} = \text{sum } \{V_A \ldots V_C\} = 3*V_{MIN} \text{ (for the array with 24 LEDs)} \quad \text{(eq 6.2)}$$

$$\text{Total } V_{MIN} = \text{sum } \{V_A \ldots V_F\} = 6*V_{MIN} \text{ (for the array with 48 LEDs)} \quad \text{(eq 6.3)}$$

The V_{TYP} and V_{MAX} are calculated in the same way as the V_{MIN}.

Table 6.5. Total electrical values for used LED-arrays. (Modified from Lavrijsen, 2003).

	I_{AF} (A)	V_{MIN} (V)	V_{TYP} (V)	V_{MAX} (V)
24 LEDs	2.8	6.93	8.85	10.53
48 LEDs	2.8	13.86	17.70	21.06

Other electronic equipment

In order to run the LED arrays in the way needed for this research some other electronic equipment was needed. In the first place the current supply to the LED arrays had to be cut off at the end of the photoperiod. Therefore a relay was made, which was capable of interrupting the current flow to the arrays. The linkage between the arrays and the relay was made by a measurement box which was already made for the measurement of the current supplied to the arrays. Another box was made whit a fuse, which would blow when the arrays where short circuited or when the current on the arrays was too high. Last but not least a box was made to turn off the current flow completely, since there always was some leakage current from the current source when it should be turned off completely.

These components will not be discussed any further here, because it is not the purpose of this research to give an in-depth review of the electronic equipment needed to drive an LED array.

Validation of the Matlab scripts

The Matlab scripts were validated by measuring the light intensity that the pulsed LED array could generate, at a given current and at points which could also be given as an output of the Matlab scripts. In the Matlab scripts a same model was introduced, which contained 48 LEDs

which were placed at the exact same locations as the real array, and were spread out over an area of approximately 300 cm^2 over a total modelled area of 500 cm^2. In total the light intensity at 121 data points was modelled and measured, then these light intensities where compared by an analysis of variance (ANOVA) test in Genstat (Version 7.1.0.205, Lawes agricultural trust, 2003).

Evaluation of the LED array

In order to check whether it was possible to get a high enough light intensity out of the array, it had to be evaluated. In order to do this, a set-up was made wherein the current and the light intensity could be measured simultaneously by passing the current through a measurement box before reaching the LED array. The current was measured with a graphical-display-meter (Voltcraft, GDM705, SN: 707120752), and light intensity measurement was done with a quantumsensor (Li-cor, Li-250). The variability in light output of the different LED arrays was measured with a quantumsensor, which was placed a few centimetres above the height where the duckweed fronds were growing. The LED arrays where set to emit the light intensities that they would normally do during an experiment, 200 and 400 µmol m^{-2} s^{-1} respectively for the continuous and pulsed LED array (see paragraph 2.3). The quantumsensor was placed at various predefined locations in the area where the aquaria with the duckweed fronds would normally be placed. In total 48 datapoints were taken in this 216 cm^2 area, from these datapoints the variability was calculated as:

$$Variability(\%) = \left(\frac{\left(X_{\max} - X_{\min} \right)}{\overline{X}} \right) * 100 \qquad \text{(eq 6.4)}$$

6.3 Results

Evaluation of the LED array

The light intensity that was measured at 10 cm from the LED array increased linearly with increasing current (Figure 6.8).

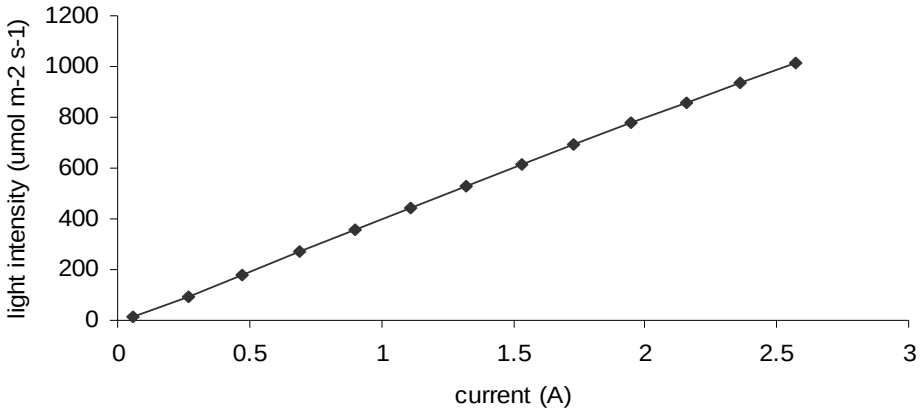

Figure 6.8. The light intensity measured at 10 cm from the LED array versus the current applied on the array.

Validation of the Matlab scripts

Although the light intensities predicted by the Matlab scripts are slightly lower then the actual intensities of the LED array, there is no significant difference (P=0.251) between them (table 6.6 and appendix H).

Table 6.6 Statistical analysis of the accuracy of the Matlab scripts used to model the amount of LEDs needed for an LED array.

Matlab scripts	LED array
44.06[1] NS[2]	46.79 NS

[1] Values given are averages over 121 datapoints.
[2] Means followed by NS do not differ significantly (*P*<5%) as established by an analysis of variance.

Variability in light output

The different variability's of the two LED arrays are given in table 6.7. For comparison the variability in the light output of the fluorescent lamps is also given in this table. The variability

percentage for the pulsed array was the highest, that for the continuous array was the lowest and the fluorescent lamps were in between (Table 6.7).

Table 6.7 Variability in the light output of the LED arrays, and of the fluorescent lamps.

Continuous array	Pulsed array	Fluorescent lamps
12.49 %	18.81 %	14.55 %

6.4 Discussion

Evaluation of the LED array

The LED arrays reacted in a way that was expected, in that the light intensity increased linearly (figure 6.8) with respect to the current applied on the arrays (Datasheet Luxeon DS23, 2003). This means that the array reacts in the same way as the single LEDs do.

Validation of the Matlab scripts

The Matlab model that was build made it possible to estimate how many LEDs should be placed to reach a certain light intensity. Furthermore it could even give an estimation of the best place to place the LEDs. However it had to be checked against reality with other arrays than that where used to build the model in the first place. Therefore when the first LED array was build, it was checked against the model to see if there where any differences. It seems that the model makes a reasonable estimate of the reality, since there was no significant difference between the model and the real LED array (table 6.6). However the small underestimation of the model can be easily explained by the fact that we were only able to measure the light intensity emitted by the LEDs at maximally 10 cm from the LED. While the LEDs will probably emit light further away, this will influence the model in estimating a lower overall light intensity.

All in all the Matlab scripts can be used to model how an LED array will react. As described in paragraph 6.2 the scripts could even be used to model the light output of other light sources, however some minor changes have to be made before it can model this. Furthermore a same type of validation is required before it can be made certain that the model works. But in conclusion it is a good model for an estimation of how much light a light source will emit and how it is spread out over a given area.

Variability in light output

The variability for the pulsed array was higher than for the continuous array, but this result is quite obvious, because the pulsed array consists of twice the amount of LEDs of the continuous array. This will lead to spots with a very high intensity and some spots that have a quite low intensity. Furthermore some errors were made during the construction of the pulsed array and the LEDs are due to these errors not placed exactly where they should be and are not dispersed the

way they should be. This will also lead to more variability in the light output. However, since the variability in light output of both LED arrays lies in the same range of that of the fluorescent lamps, the variability will probably not lead to much difference in the experiments. However it is something to keep an eye on, certainly when the aquaria filled with the duckweed fronds are always placed at the same location.

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Appendix A: Analysis of the nutrient solution used

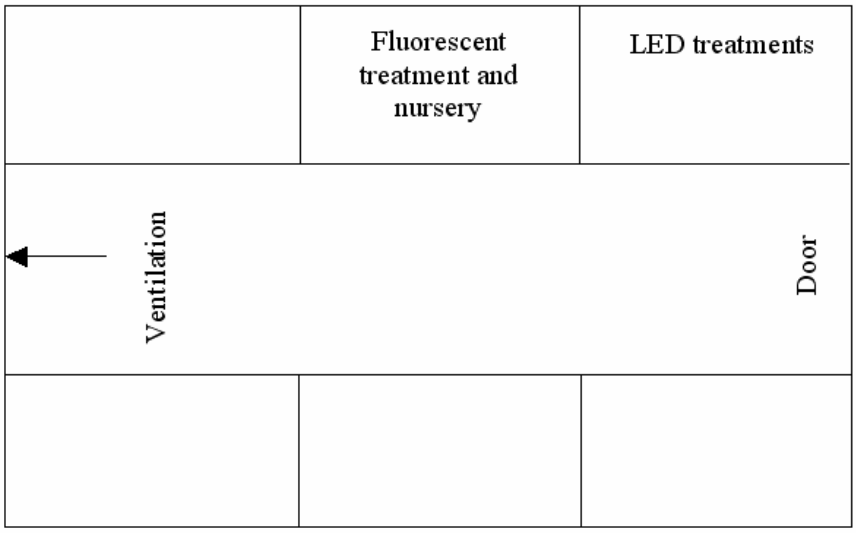
Amount of nutrients supplied per nutrient supply level

Nutrient supply level	Complete Nutrients	Half Nutrients
Macro elements (mmol l^{-1})		
N	14.79	7.40
P	1.78	0.89
K	13.85	6.93
S	5.66	2.83
Ca	5.56	2.78
Mg	2.52	1.26
Trace elements (μmol l^{-1})		
Fe	25.0	12.5
Mn	10.0	5.0
Zn	5.0	2.5
B	40.0	20.0
Cu	1.0	0.5
Mo	0.5	0.3
Cl	2.1	1.1

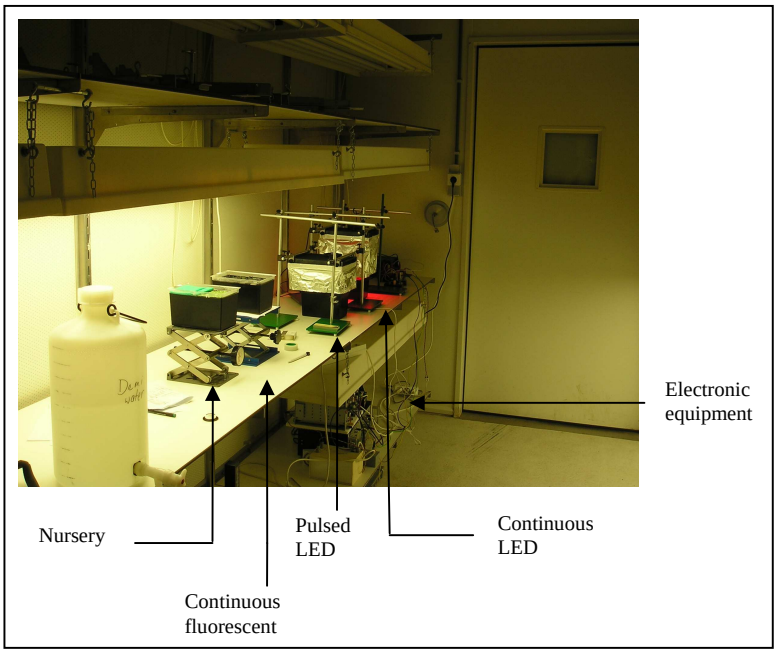
Appendix B: Drying procedure of the oven

1. Warming up till 70°C
2. 3 hours at 70°C
3. Warming up till 105°C
4. 10 hours at 105°C
5. Cooling off till 70°C
6. 2 hours at 70°C

Appendix C: Set-up of the Lemna experiments in the climate chamber



Top view of the climate chamber with the set-up of the different Lemna treatments.



In line view of the the climate chamber with the set-up of the different Lemna treatments

Appendix D: Analysis of variance Spirodela experiment

Analysis of variance for the variates, average area occupied by Spirodela fronds and dry_weight of the Spirodela fronds. Abbreviations: d.f.= degrees of freedom, s.s.= sum of squares, m.s.= mean sum of squares, v.r.= variance ratio, F pr.= F probabillity, rep.= repetitions, l.s.d.= least significant difference, HN= half nutrient solution half demineralised water, CN= complete nutrient solution

Variate: average_area

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
light_period	1	7.224	7.224	2.22	0.146
nutrient_treatment	1	0.004	0.004	0.00	0.973
light_period.nutrients	1	0.309	0.309	0.09	0.760
Residual	32	104.354	3.261		
Total	35	111.891			

***** Tables of means *****

Grand mean 2.49

light_period	16	24
	2.05	2.94

nutrient_treatment	HN	CN
	2.50	2.48

light_period	nutrient_treatment	HN	CN
16		2.15	1.94
24		2.86	3.02

*** Least significant differences of means (5% level) ***

Table	light_period	nutrient_treatment	light_period Nutrient_treatment
rep.	18	18	9
d.f.	32	32	32
l.s.d.	1.226	1.226	1.734

Variate: average_area

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
light_source	1	1.275	1.275	1.23	0.284
Residual	16	16.577	1.036		
Total	17	17.852			

***** Tables of means *****

Grand mean 1.68

light_source	LED	Fluorescent
	1.41	1.94

*** Least significant differences of means (5% level) ***

Table	light_source
rep.	9
d.f.	16
l.s.d.	1.017

Variate: dry_weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
light_period	1	0.5852	0.5852	0.98	0.342
nutrient_treatment	1	0.1089	0.1089	0.18	0.677

```
light_period.nutrient_treatment
                          1     0.3192     0.3192    0.53  0.479
Residual                 12     7.1875     0.5990
Total                    15     8.2009

***** Tables of means *****

Grand mean  0.76

 light_period       24       16
                  0.95     0.57

 nutrient_treatment       CN       HN
                        0.84     0.68

 light_period nutrient_treatment       CN       HN
           24                        1.18     0.73
           16                        0.51     0.63

*** Least significant differences of means (5% level) ***

Table          light_period nutrient_treatment light_period
                                                nutrient_treatment
rep.                      8           8           4
d.f.                     12          12          12
l.s.d.                0.843       0.843       1.192
```

Variate: dry_weight

```
Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
light_source               1     0.0015     0.0015    0.01  0.932
Residual                   6     1.1569     0.1928
Total                      7     1.1584

***** Tables of means *****

Grand mean  0.53

 light_source         LED Fluorescent
                     0.54        0.51

*** Least significant differences of means (5% level) ***

Table          light_source
rep.                      4
d.f.                      6
l.s.d.                0.760
```

Appendix E: statistical analysis of theLemna experiments

Experiment 1 (1.28 Hz)

Variate: DW_at_day_0_mg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	0.0002170	0.0001085	0.80	0.458
Residual	33	0.0044804	0.0001358		
Total	35	0.0046973			

* MESSAGE: the following units have large residuals.

units 15	0.0257	s.e. 0.0112
units 18	0.0278	s.e. 0.0112
units 32	0.0298	s.e. 0.0112

***** Tables of means *****

Variate: DW_at_day_0_mg

Grand mean 0.0457

Treatment	continuous fluorescent	continuous LED	pulsed LED
	0.0488	0.0453	0.0429

*** Least significant differences of means (5% level) ***

Table	Treatment
rep.	12
d.f.	33
l.s.d.	0.00968

Variate: dry_weight_g

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	3.005E-05	1.502E-05	2.86	0.073
Residual	30	1.576E-04	5.255E-06		
Total	32	1.877E-04			

* MESSAGE: the following units have large residuals.

units 14	0.00602	s.e. 0.00219

***** Tables of means *****

Variate: dry_weight_g

Grand mean 0.00812

Treatment	continuous fluorescent	continuous LED	pulsed LED
	0.00942	0.00778	0.00715

*** Least significant differences of means (5% level) ***

Table	Treatment
rep.	11
d.f.	30
l.s.d.	0.001996

Variate: relative_growth_rate

```
Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
treatment                  2  0.0080328  0.0040164    9.71  <.001
Residual                  33  0.0136458  0.0004135
Total                     35  0.0216786

* MESSAGE: the following units have large residuals.

*units* 16         0.0485   s.e. 0.0195
*units* 19        -0.0426   s.e. 0.0195

***** Tables of means *****

Variate: relative_growth_rate

Grand mean  0.3533

 Treatment continuous fluorescent            continuous LED         pulsed LED
                           0.3722                    0.3357             0.3520

 *** Least significant differences of means (5% level) ***

Table            treatment
rep.                    12
d.f.                    33
l.s.d.             0.01689
```

Variate: LAR_cm2_mg

```
Source of variation     d.f.(m.v.)      s.s.       m.s.    v.r.  F pr.
Treatment                    2          2.4447     1.2223   12.17  <.001
Residual                  30(3)         3.0123     0.1004
Total                     32(3)         5.2533

* MESSAGE: the following units have large residuals.

*units* 27          0.806   s.e. 0.289
*units* 34         -0.637   s.e. 0.289

***** Tables of means *****

Variate: LAR_cm2_mg

Grand mean  3.189

 Treatment continuous fluorescent         continuous LED              pulsed LED
                           3.288                  2.832                   3.447

*** Least significant differences of means (5% level) ***

Table            Treatment
rep.                    12
d.f.                    30
l.s.d.              0.2642

(Not adjusted for missing values)
```

Experiment 2 (1.28 Hz-2)

Variate: DW_day_0

```
Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
Treatment                  2 0.00003590 0.00001795    0.45  0.642
```

```
Residual                  33 0.00131871 0.00003996
Total                     35 0.00135461

* MESSAGE: the following units have large residuals.

*units* 6          0.0160   s.e. 0.0061

***** Tables of means *****

Variate: DW_day_0

Grand mean  0.0296

 Treatment continuous fluorescent         continuous LED             pulsed LED
                           0.0284                 0.0308                 0.0295

*** Least significant differences of means (5% level) ***

Table            Treatment
rep.                    12
d.f.                    33
l.s.d.             0.00525
```

Variate: dry_weight_mg

```
Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
Treatment                  2    0.00257    0.00129    0.13  0.880
Residual                  33    0.33059    0.01002
Total                     35    0.33316


* MESSAGE: the following units have large residuals.

*units* 6           0.240   s.e. 0.096
*units* 10          0.230   s.e. 0.096


***** Tables of means *****

Variate: dry_weight_mg

Grand mean  0.412

 Treatment continuous fluorescent         continuous LED             pulsed LED
                           0.400                  0.418                  0.417


*** Least significant differences of means (5% level) ***

Table            Treatment
rep.                    12
d.f.                    33
l.s.d.              0.0831
```

Variate: relative_growth_rate

```
Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
treatment                  2  0.0112479  0.0056240   15.08  <.001
Residual                  33  0.0123068  0.0003729
Total                     35  0.0235548

* MESSAGE: the following units have large residuals.

*units* 4          -0.0564   s.e. 0.0185

 ***** Tables of means *****

Variate: relative_growth_rate
```

```
Grand mean  0.3069

 Treatment continuous fluorescent      continuous LED       pulsed LED
                          0.3266              0.2837           0.3105

*** Least significant differences of means (5% level) ***

Table            treatment
rep.                    12
d.f.                    33
l.s.d.             0.01604


Variate: LAR_cm2_mg

Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
Treatment                  2     5.5601     2.7801    6.25  0.005
Residual                  33    14.6849     0.4450
Total                     35    20.2450

* MESSAGE: the following units have large residuals.

*units* 3            3.02   s.e. 0.64

***** Tables of means *****

Variate: LAR_cm2_mg

Grand mean  4.19

 Treatment continuous fluorescent         continuous LED             pulsed LED
                             4.61                   3.66                   4.29

*** Least significant differences of means (5% level) ***

Table            Treatment
rep.                    12
d.f.                    33
l.s.d.               0.554
```

Experiment 3 (0.12 Hz)

```
Variate: DW_day_0

Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
Treatment                  2 0.00003647 0.00001824    0.70  0.502
Residual                  33 0.00085592 0.00002594
Total                     35 0.00089240

* MESSAGE: the following units have large residuals.

*units* 30        0.01255   s.e. 0.00488

***** Tables of means *****

Variate: DW_day_0

Grand mean  0.03033

 Treatment continuous fluorescent         continuous LED             pulsed LED
                          0.02976                0.02948                0.03174

*** Least significant differences of means (5% level) ***

Table            Treatment
rep.                    12
```

```
d.f.                    33
l.s.d.            0.004230
```

Variate: dry_weight_mg

```
Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
Treatment                  2    0.29207    0.14603   14.09  <.001
Residual                  33    0.34201    0.01036
Total                     35    0.63407

* MESSAGE: the following units have large residuals.

*units* 17          0.222   s.e. 0.097

***** Tables of means *****

Variate: dry_weight_mg

Grand mean  0.497

 Treatment continuous fluorescent         continuous LED             pulsed LED
                            0.516                  0.598                  0.379

*** Least significant differences of means (5% level) ***

Table            Treatment
rep.                    12
d.f.                    33
l.s.d.              0.0846
```

Variate: relative_growth_rate

```
Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
Treatment                  2  0.0151689  0.0075844   28.97  <.001
Residual                  33  0.0086403  0.0002618
Total                     35  0.0238091

* MESSAGE: the following units have large residuals.

*units* 8         -0.0354   s.e. 0.0155
*units* 31        -0.0402   s.e. 0.0155

***** Tables of means *****

Variate: relative_growth_rate

Grand mean  0.3421

Treatment  continuous fluorescent            continuous LED                pulsed LED
                        0.3502                    0.3621                    0.3139

*** Least significant differences of means (5% level) ***

Table            Treatment
rep.                    12
d.f.                    33
l.s.d.             0.01344
```

Variate: LAR_cm2_mg

```
Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
Treatment                  2     2.0150     1.0075    4.12  0.025
Residual                  33     8.0628     0.2443
Total                     35    10.0778

***** Tables of means *****
```

```
Variate: LAR_cm2_mg

Grand mean  4.188

 Treatment continuous fluorescent         continuous LED             pulsed LED
                            4.206                  3.890                  4.469

*** Least significant differences of means (5% level) ***

Table            Treatment
rep.                    12
d.f.                    33
l.s.d.              0.4106
```

Experiment 4 (12 Hz)

Variate: DW_day_0

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	0.00015376	0.00007688	5.01	0.013
Residual	33	0.00050605	0.00001533		
Total	35	0.00065981			

```
* MESSAGE: the following units have large residuals.

*units* 28        0.01035   s.e. 0.00375

***** Tables of means *****

Variate: DW_day_0

Grand mean  0.02297

 Treatment continuous fluorescent         continuous LED             pulsed LED
                           0.02579                0.02221                0.02090

*** Least significant differences of means (5% level) ***

Table            Treatment
rep.                    12
d.f.                    33
l.s.d.             0.003253
```

Variate: Dry_weight_mg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	0.21740	0.10870	9.97	<.001
Residual	33	0.35988	0.01091		
Total	35	0.57727			

```
* MESSAGE: the following units have large residuals.

*units* 11          0.236   s.e. 0.100
*units* 28          0.256   s.e. 0.100

***** Tables of means *****

Variate: Dry_weight_mg

Grand mean  0.436

 Treatment continuous fluorescent         continuous LED             pulsed LED
                           0.534                  0.429                  0.344

*** Least significant differences of means (5% level) ***
```

```
Table           Treatment
rep.                   12
d.f.                   33
l.s.d.            0.0867
```

Variate: relative_growth_rate

```
Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
Treatment                  2  0.0122897  0.0061449   30.87  <.001
Residual                  33  0.0065687  0.0001991
Total                     35  0.0188584

***** Tables of means *****

Variate: relative_growth_rate

Grand mean  0.3470

Treatment continuous fluorescent         continuous LED             pulsed LED
                          0.3731                 0.3343                 0.3335

*** Least significant differences of means (5% level) ***

Table           Treatment
rep.                   12
d.f.                   33
l.s.d.            0.01172
```

Variate: LAR_cm2_mg

```
Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
Treatment                  2     2.7909     1.3954    9.07  <.001
Residual                  33     5.0786     0.1539
Total                     35     7.8695

* MESSAGE: the following units have large residuals.

*units* 18          0.916   s.e. 0.376
*units* 30          0.881   s.e. 0.376

***** Tables of means *****

Variate: LAR_cm2_mg

Grand mean  4.294

 Treatment continuous fluorescent         continuous LED             pulsed LED
                           4.662                  3.988                  4.231

*** Least significant differences of means (5% level) ***

Table           Treatment
rep.                   12
d.f.                   33
l.s.d.            0.3258
```

Experiment 5 (0.012 Hz)

Variate: DW_day_0

```
Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
Treatment                  2 0.00018380 0.00009190    2.13  0.135
Residual                  33 0.00142540 0.00004319
Total                     35 0.00160920
```

```
***** Tables of means *****

Variate: DW_day_0

Grand mean  0.0386

 Treatment continuous fluorescent         continuous LED             pulsed LED
                           0.0377                 0.0418                 0.0365

*** Least significant differences of means (5% level) ***

Table            Treatment
rep.                    12
d.f.                    33
l.s.d.             0.00546
```

Variate: Dry_weight_mg

```
Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
Treatment                  2    2.84715    1.42358   91.34  <.001
Residual                  33    0.51433    0.01559
Total                     35    3.36148

* MESSAGE: the following units have large residuals.

*units* 16          0.368   s.e. 0.120

***** Tables of means *****

Variate: Dry_weight_mg

Grand mean  0.674

 Treatment continuous fluorescent         continuous LED             pulsed LED
                           0.884                 0.862                 0.277

*** Least significant differences of means (5% level) ***

Table            Treatment
rep.                    12
d.f.                    33
l.s.d.              0.1037
```

Variate: relative_growth_rate

```
Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
Treatment                  2  0.0990136  0.0495068  395.18  <.001
Residual                  33  0.0041341  0.0001253
Total                     35  0.1031477

* MESSAGE: the following units have large residuals.

*units* 23        -0.0307   s.e. 0.0107
*units* 24        -0.0270   s.e. 0.0107

***** Tables of means *****

Variate: relative_growth_rate

Grand mean  0.3263

 Treatment continuous fluorescent         continuous LED             pulsed LED
                           0.3736                 0.3520                 0.2531

*** Least significant differences of means (5% level) ***

Table            Treatment
rep.                    12
```

```
d.f.                    33
l.s.d.             0.00930
```

Variate: LAR_cm2_mg

```
Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
Treatment                  2    15.0940     7.5470   41.49  <.001
Residual                  33     6.0031     0.1819
Total                     35    21.0972

* MESSAGE: the following units have large residuals.

*units* 29          1.223   s.e. 0.408

***** Tables of means *****

Variate: LAR_cm2_mg

Grand mean  4.861

 Treatment continuous fluorescent         continuous LED             pulsed LED
                            4.690                  4.168                  5.726

*** Least significant differences of means (5% level) ***

Table            Treatment
rep.                    12
d.f.                    33
l.s.d.              0.3543
```

Experiment 6 (120 Hz)

Variate: DW_day_0

```
Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
Treatment                  2 0.00009976 0.00004988    1.10  0.346
Residual                  33 0.00150098 0.00004548
Total                     35 0.00160074

* MESSAGE: the following units have large residuals.

*units* 18         0.0161   s.e. 0.0065
*units* 36         0.0152   s.e. 0.0065

***** Tables of means *****

Variate: DW_day_0

Grand mean  0.0448

 Treatment continuous fluorescent         continuous LED             pulsed LED
                          0.0435                 0.0471                 0.0437

*** Least significant differences of means (5% level) ***

Table            Treatment
rep.                    12
d.f.                    33
l.s.d.             0.00560
```

Variate: dry_weight_mg

```
Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
Treatment                  2    0.11832    0.05916    2.57  0.092
Residual                  33    0.76088    0.02306
```

```
Total                     35    0.87920

***** Tables of means *****

Variate: dry_weight_mg

Grand mean  0.900

 Treatment continuous fluorescent         continuous LED             pulsed LED
                            0.946                  0.935                  0.819

*** Least significant differences of means (5% level) ***

Table            Treatment
rep.                    12
d.f.                    33
l.s.d.              0.1261
```

Variate: relative_growth_rate

```
Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
Treatment                  2  0.0067685  0.0033842   23.39  <.001
Residual                  33  0.0047756  0.0001447
Total                     35  0.0115441


* MESSAGE: the following units have large residuals.

*units* 16        -0.0267   s.e. 0.0115


***** Tables of means *****

Variate: relative_growth_rate

Grand mean  0.3423

 Treatment continuous fluorescent         continuous LED             pulsed LED
                           0.3611                 0.3372                 0.3287


*** Least significant differences of means (5% level) ***

Table            Treatment
rep.                    12
d.f.                    33
l.s.d.             0.00999
```

Variate: LAR_cm2_mg

```
Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
Treatment                  2    3.09471    1.54736   36.35  <.001
Residual                  33    1.40489    0.04257
Total                     35    4.49960

* MESSAGE: the following units have large residuals.

*units* 2           0.594   s.e. 0.198
*units* 10         -0.544   s.e. 0.198

***** Tables of means *****

Variate: LAR_cm2_mg

Grand mean  3.940

 Treatment continuous fluorescent         continuous LED             pulsed LED
                           4.343                  3.825                  3.653
```

*** Least significant differences of means (5% level) ***

Table	Treatment
rep.	12
d.f.	33
l.s.d.	0.1714

Appendix F: statistical analysis of the Pulsing comparison

ANOVA of the linear trends

RAGR

```
Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
treatment                  1  0.0010665  0.0010665    3.23  0.103
Residual                  10  0.0033068  0.0003307
Total                     11  0.0043734

* MESSAGE: the following units have large residuals.

*units* 7         -0.0346   s.e. 0.0166
*units* 12         0.0342   s.e. 0.0166

***** Tables of means *****

Variate: estimate

Grand mean  0.3248

 treatment continuous     pulsed
              0.3342     0.3153

*** Least significant differences of means (5% level) ***

Table            treatment
rep.                     6
d.f.                    10
l.s.d.             0.02339
```

LAR

```
Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
treatment                  1     0.9864     0.9864    4.98  0.050
Residual                  10     1.9791     0.1979
Total                     11     2.9655

* MESSAGE: the following units have large residuals.

*units* 7            0.89   s.e. 0.41
*units* 12          -0.88   s.e. 0.41

***** Tables of means *****

Variate: estimate

Grand mean  4.03

 treatment continuous     pulsed
                3.74       4.31

*** Least significant differences of means (5% level) ***


Table            treatment
rep.                     6
d.f.                    10
l.s.d.               0.572
```

Continuous fluorescent

Variate: LAR_cm2_mg

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
frequency_Hz	5	16.9612	3.3922	12.82	<.001
Residual	65(1)	17.1983	0.2646		
Total	70(1)	33.1216			

```
* MESSAGE: the following units have large residuals.

*units* 39          3.018   s.e. 0.489
```

***** Tables of means *****

Variate: LAR_cm2_mg

Grand mean 4.300

frequency_Hz	0.012	0.12	1.28 (1)	1.28 (2)	12	120
	4.690	4.206	3.288	4.610	4.662	4.343

*** Least significant differences of means (5% level) ***

Table	frequency_Hz
rep.	12
d.f.	65
l.s.d.	0.4194

Variate: RAGR_cm2_cm_2_day_1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
frequency_Hz	5	0.0206006	0.0041201	14.69	<.001
Residual	66	0.0185108	0.0002805		
Total	71	0.0391113			

```
* MESSAGE: the following units have large residuals.

*units* 27         0.0406   s.e. 0.0160
*units* 40        -0.0564   s.e. 0.0160
```

***** Tables of means *****

Variate: RAGR_cm2_cm_2_day_1

Grand mean 0.3595

frequency_Hz	0.012	0.12	1.28 (1)	1.28 (2)	12	120
	0.3736	0.3502	0.3722	0.3266	0.3731	0.3611

*** Least significant differences of means (5% level) ***

Table	frequency_Hz
rep.	12
d.f.	66
l.s.d.	0.01365

Continuous LED

Variate: LAR_cm2_mg

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
frequency_Hz	5	13.2478	2.6496	25.71	<.001
Residual	65(1)	6.6993	0.1031		

```
Total                     70(1)      19.1334

* MESSAGE: the following units have large residuals.

*units* 20          0.746   s.e. 0.305
*units* 54          0.916   s.e. 0.305

***** Tables of means *****

Variate: LAR_cm2_mg

Grand mean  3.728

 frequency_Hz    0.012     0.12 1.28 (1) 1.28 (2)       12      120
                 4.168    3.890    2.832    3.663    3.988    3.825

*** Least significant differences of means (5% level) ***

Table          frequency_Hz
rep.                    12
d.f.                    65
l.s.d.              0.2618
```

Variate: RAGR_cm2_cm_2_day_1

```
Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
frequency_Hz               5  0.0438496  0.0087699   34.25  <.001
Residual                  66  0.0169006  0.0002561
Total                     71  0.0607502

* MESSAGE: the following units have large residuals.

*units* 28         0.0485   s.e. 0.0153
*units* 31        -0.0426   s.e. 0.0153

***** Tables of means *****

Variate: RAGR_cm2_cm_2_day_1

Grand mean  0.3342

 frequency_Hz    0.012     0.12 1.28 (1) 1.28 (2)       12      120
                0.3520   0.3621   0.3357   0.2837   0.3343   0.3372

*** Least significant differences of means (5% level) ***

Table          frequency_Hz
rep.                    12
d.f.                    66
l.s.d.             0.01304
```

Pulsed LED

Variate: LAR_cm2_mg

```
Source of variation     d.f.(m.v.)      s.s.       m.s.    v.r.  F pr.
frequency_Hz               5          38.5648     7.7130   34.94  <.001
Residual                  65(1)       14.3490     0.2208
Total                     70(1)       52.1723

* MESSAGE: the following units have large residuals.

*units* 5           1.223   s.e. 0.446

***** Tables of means *****
```

```
Variate: LAR_cm2_mg

Grand mean  4.302

 frequency_Hz    0.012     0.12 1.28 (1) 1.28 (2)       12      120
                 5.726    4.469    3.447    4.286    4.231    3.653

*** Least significant differences of means (5% level) ***

Table          frequency_Hz
rep.                   12
d.f.                   65
l.s.d.             0.3831
```

Variate: RAGR_cm2_cm_2_day_1

```
Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
frequency_Hz               5  0.0689377  0.0137875   62.07  <.001
Residual                  66  0.0146600  0.0002221
Total                     71  0.0835978

* MESSAGE: the following units have large residuals.

*units* 19        -0.0402   s.e. 0.0143

***** Tables of means *****

Variate: RAGR_cm2_cm_2_day_1

Grand mean  0.3153

 frequency_Hz    0.012     0.12 1.28 (1) 1.28 (2)       12      120
                0.2531   0.3139   0.3520   0.3105   0.3335   0.3287

*** Least significant differences of means (5% level) ***

Table          frequency_Hz
rep.                   12
d.f.                   66
l.s.d.            0.01215
```

Appendix G: Matlab scripts.

Red.m

```
% first step is to input the size of the square grid within which the leds will be
located
% and the irradiance calculated
grid_size=input('input the length of the side of the square of grid\n')
% next input the array of vectors (co-ords) of the led locations
led_locations=input('input a vector of led locations as [a b; c d; e f; etc]\n')
% calculate the loop number (number of led locations input)
loop_number=length(led_locations);
%make a list of serial numbers corresponding to every cell in the grid
grid_cells=make_list(grid_size);
%pre_allocate space for output array of irradiances - needs to have a length equal to the
number of cells in the grid
% and to have a width equal to the number of led locations
irr_array=zeros(grid_size^2, loop_number);
% begin loop procedure to calculate for every led location a distance from every cell in
the grid and the corresponding
%irradiance at that cell in the grid
for location = 1:loop_number
      distance=index_vec(grid_cells, grid_size, led_locations(location,:)); (-)
   distance(:,2)=(7*distance(:,2)); (*)
% calculate the irradiance based on the distances
light_intensity=(-0.0021*(distance(:,2).^2))+(0.0179.*distance(:,2))+6.8674; (*)
%eliminate any negative values of irradiance
neg=find(light_intensity<0);
light_intensity(neg)=zeros(size(neg));
%place irradiance values into the output array (pre-allocated)
irr_array(:,location)=light_intensity;
end
% calculate the sum of the different irradiance values
x = sum(irr_array,2); (-)
% Reshape the sum of the irradiance values in order to view them
y = reshape(x,grid_size,grid_size);
% show the amount of LEDS present
loop_number
% Show the irradiance plot
subplot(1,2,1);image(y); colormap(hot); title('Irradiance plot')
% plot the places of the LEDs
subplot(1,2,2); plot(led_locations(:,1),led_locations(:,2), '*'); title('LED positions');
% show diversity
diversity
```

make_list.m

```
function list_of_numbers=make_list(square_size);
%input a number, the length of a side of a square, and this function returns a list of
numbers whose length matches the
%number of cells in the grid
list_of_numbers=1:square_size^2;
list_of_numbers=list_of_numbers';
```

index_vec.m

```
function distance = index_vec(list, grid_size, ref_points);
% inputs: list - a list of numbers of a length equal to the number of cells in a grid
% inputs: grid_size the length of a side of the grid
% inputs: ref_points the points from which the light gradient will be calculated
% next: make an array of vectors for each cell in the grid this is vec_array
% first column contains the row elements - should be like 1 2 3 4 etc
vec_array(:,1)=list./grid_size;
vec_array(:,1)=ceil(vec_array(:,1));
vec_array(:,1) = vec_array(:,1)-1;
vec_array(:,1)=list-( grid_size.*vec_array(:,1));
%second column contains the column addresses should be like 1 1 1 1
vec_array(:,2)=list./grid_size;
vec_array(:,2)=ceil(vec_array(:,2));
% now calculate distance between each point referred to in vec_array and ref_point
distance(:,1)=(((vec_array(:,1)-ref_points(:,1)).^2)+((vec_array(:,2)-
ref_points(:,2)).^2)).^0.5;
distance=[list distance];
```

Diversity.m

```
% DIVERSITY.M Creates variables which can be called upon to check if everything is still
ok, it is a subroutine of
% the irradiation calulations. These variables can be used to plot a line function to
asses the variability
% First the column with the maximum values of irradiance is chosen and named cat
cat=max(y);
% Next from this column the maximum value is chosen and again named cat
cat=max(cat);
% Next the irradiance is divided by the maximal vlaue in order to get a fraction of the
maximum the output is called dog
dog=y./cat;
% lastly the fraction is converted into a percentage by multiplying with 100, this
variable is called z
z=100.*dog;
% The variable z needs to be plotted against something, and this will be the grid size so
a list of 1 till grid size is
% made and shaped in one column in stead of in one row, this variable is called number.
number=[1:grid_size];
number=number';
```

Appendix H: Analysis of variance Matlab scripts

Abbreviations: mat: matlab scripts, mea: measured from LED array.

Variate: light_intensity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	1	448.8	448.8	1.32	0.251
Residual	240	81501.7	339.6		
Total	241	81950.5			

***** Tables of means *****

Grand mean 45.4

treatment	mat	mea
	44.1	46.8

*** Least significant differences of means (5% level) ***

Table	treatment
rep.	121
d.f.	240
l.s.d.	4.67