Photosynthesis model to predict duckweed growth at the Ecoferm greenhouse

Chair group Biobased Chemistry & Technology
BSc Thesis Biosystems Engineering

Pieter Rooijakkers
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Student	: Pieter Rooijakkers
Registration number	: 950407708120
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Supervisor	: Dr. R.J.C. van Ooteghem
Examiner	: Dr. ir. A.J.B. van Boxtel

Abstract

The Ecoferm greenhouse located in Uddel (the Netherlands) contains a pond on top of a calves stable where duckweed is cultivated. Operating in a green cycle where the calves manure (thin fraction), urea and CO_2 is used for duckweed growth and after all the duckweed is used again for feeding the calves. Furthermore, the thick fraction is used to feed three mono-digesters, which leads to a full self-sufficiency of electricity. In this report, only the duckweed pond is discussed.
Unfortunately for the duckweed cultivation, circumstances occurred that were fatal for the duckweed, which lead to a change in the dominant species and slight modifications to the pond. First, the Lemna minor (lesser duckweed) was cultivated as dominant species with an addition of the Spirodel polyrhiza (giant duckweed) species. After a period where the temperature increased to lethal situations, the breeding culture was switched from dominant species.

The biggest differences between the two duckweed species that have been used on the Ecoferm greenhouse are the temperature tolerance, light intensity saturation and the minimum concentration of oxygen available in the pond. Furthermore, there are physical differences in frond size and root number.

In this report, the input data for the light intensity is approached more accurate than former research using a photosynthesis model. In this model, other inputs like CO_2 concentration, temperature and pH level were used to predict the growth of duckweed at Ecoferm for the months May, September and October. The data of harvest and input parameters for the remaining months in the cultivation period was not accurate enough for validation. This model is described in chapter 4. To gain more knowledge about how the photosynthetic processes work and what the differences are between the two duckweed species used at the Ecoferm, read chapter 2.

The results of the photosynthesis model for the months discussed above are compared with real harvest data and the aim is to give approximate this data as good as possible using the programming software MathWorks MATLAB R2016a.

The optimal growth parameters were determined using parameter estimation and a fisher information to indicate the parameter values and the parameter sensitivity respectively. The following parameters were used for the parameter estimation (5 of the 10 parameters used for the approach of the duckweed growth, the contents of each parameter can be found in Table 7):

- J_{max26}
- R_s
- Γ
- θ
- R_{cut}

The parameters were tweaked in a way that the model suited all the months well. Performing the model with the estimated parameters gives the following results:

Total production of each month (kg). Note: the last day of the harvest data corresponding the month May is not equal to the last day of the month. The model data is compared with the last day of the harvest data.

Month	Original (kg)	Fit (kg)	Harvest data (kg)	Deviation original	Deviation fit	Improvement
May	1223.951	1653.053	1941.667	37%	15%	22%
September	675.141	1054.113	1220.000	45%	14%	31%
October	389.433	581.904	640.000	39%	9%	30%

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1. Introduction

1.1 Background

A big issue in Dutch livestock is the manure surplus they produce. Formerly, all of the manure produced could be distributed over the fields and also pigs and calves farmers could get rid of their manure easily. However, due to environmental issues and political pressure, huge changes took place. Intensive farmers like for example pig or calve farmers have to make big investments in manure processing and cannot deposit their surplus to dairy farmers anymore. Also, after the cancelation of the milk quota in the Netherlands, dairy farmers started to increase their livestock, which resulted in even more manure. A new policy for phosphate will come into effect on January 1, 2017, which will limit the amount of phosphate a farmer is allowed to produce up to a so called 'phosphate ceiling'. Until 2018, farmers have the opportunity to reduce this ceiling. Reduction is important for the condition the European Commission sets for maintaining derogation. This means that farmers are allowed to use more nitrogen from the produced manure on their fields, which ensures savings on manure processing (Jansen, 2016). Having more restrictions on manure use means that, also in the dairy sector, fertilization with manure is limited and manure processing has to take place. Ecoferm in Uddel (the Netherlands) is a rosé calves breeding farm that comes with a sustainable solution that ensures that the produced manure can be reused for the growth of duckweed, which in its way serves as food for the rosé calves on the same farm. Duckweed has high protein levels and can be a good alternative for soybean. Furthermore, a digester is used to produce energy from manure.

1.2 Problem description

Growth rate factors of duckweed at Ecoferm have already been researched and discussed by van den Top (2014) and Ruigrok (2015). Factors like nutrient uptake, assimilation, transport, photosynthesis, respiration and enzymatic activity play a role in the growth rate of duckweed (Landolt et al., 1987). The photosynthetic rate has a linear relation with light intensity (Peeters et al., 2013). For photosynthesis there is a minimum threshold of light intensity to start and a saturation point (600 $\mu mol/m^2s$, at 30°C, 300 $\mu mol/m^2s$) (Landolt et al., 1987). However, it is not necessary to have a light intensity near the saturation point, which means that a plant can perform photosynthesis at light intensities higher than its threshold. After increasing the intensity above 200 $\mu mol/m^2s$, the growth rate of duckweed is hardly affected. Ashby and Oxley (1935) researched the growth rate of duckweed (L. minor) as function of light intensity and temperature. They found that in general, a higher temperature and light intensity resulted in a higher growth rate (optimal: growth rate of 0.29k (where k is the growth rate in dependence of the light intensity) at 16000 lux and 29°C). In the research of Ashby and Oxley, the light intensity is kept constant for a certain period of time. Lasfar et al. (2007) used a method where a light source of 400 W and average light intensity of 371 $\mu mol/m^2s$ (±10 $\mu mol/m^2s$) (higher than saturation point) was used on a setup used to research duckweed (L. minor). In three days, the exposure time was varied between 2 and 20 hours.
At Ecoferm, the duckweed is hardly exposed to constant rates of light intensity. At present, no artificial lighting of the duckweed is used, so the light irradiation is only from the sun. In the Netherlands, where the Ecoferm is located (Uddel), most of the days are cloudy, which results in a varying light intensity. Sunlight shining through clouds causes a scattering of the rays. This means that the sunlight is converted from direct to diffuse light and is distributed in all directions. Van den Top (2014) adapted a model of Slegers et al. (2011) for the situation in Uddel and found that the diffuse and direct radiation on the roof surface (year round) has a ratio of about 50/50. A good model to predict the production of duckweed with dynamic environmental changes is not yet found. When a model can be created that

predicts the yield of duckweed under environmental influences, it can be used to make assumptions for the future on which the farmer can then anticipate.

1.3 Aim

After this research there will be more insight in how the growth rate of duckweed is affected using a photosynthesis model. The findings of this research will be compared with the available production of duckweed at the Ecoferm greenhouse. The aim is to reach a production curve that is close to the available harvest data, so that the model can be used to predict the production of duckweed with the influence of four inputs:

- Light intensity
- Temperature
- CO_2 concentration
- pH

1.4 Research questions

To reach a better prediction of the duckweed growth with the influence of the available inputs *temperature, light intensity, CO_2 concentration* and *pH* than the already available model formulated by van den Top (2014) the following questions will help to obtain the desired outcomes:

How does a photosynthesis model simulation result the production of duckweed produced on Ecoferm?

With sub questions:

1. How can we implement the available inputs into the model?
2. How can we validate the model?
3. Does the model represent the actual production well?

1.5 Delimitations

Ruigrok (2015) already researched temperature influences on duckweed, so this will not be investigated, and values about temperature conditions (such as optimal or maximum temperature) will be assumed from his thesis. Also effects of nutrients available in water will be obtained from earlier research and will be assumed constant for the whole cultivating period.
Van den Top (2014) already stated that the Ecoferm has the following subsystems: stable, manure pit, calves, bio-bed, greenhouse, mono-digester, buffers and generator. Only the pond where the duckweed is cultivated is of importance on this research.

1.6 Approach

First, a literature research will be done on the duckweed species that are cultivated at the Ecoferm greenhouse. In this research the basics of photosynthesis will be discussed because a photosynthesis model will be used to predict the duckweed growth. Then, when knowledge about these topics is mastered, a model will be made using MATLAB R2016a. The model contains several photosynthetic processes that use the available input data. At the Ecoferm greenhouse two species of Lemnaceae (duckweed) are used: Spirodela polyrhiza and Lemna minor. These two species differ from each other,

which has an effect on the growth. These differences will be discussed in the second literature part. After this, the model results will be compared with the data of the production of duckweed at the Ecoferm that is available from harvest logging. Sensitivity analysis is used to determine which parameters are most important for the model and to what extent parameter estimation has to be used. The growth before and after the parameter estimation will be shown and will be discussed if a desirable improvement is reached.

The final chapters ‘Results’ and ‘Discussion’ describe and discuss the results from the steps above. The findings are concluded in the chapter ‘Conclusions’ and some recommendations for the future will be made in the chapter ‘Recommendations’.

2. Literature

2.1. Species differences

2.1.1. Ecoferm situation

In 2014, at the start of the Ecoferm project, a mixture of two species of duckweed was installed. Lemna minor was the dominant species in the experiment. However, as van den Top (2014) and Ruigrok (2015) already discussed, Lemna minor will die after a period of lack of oxygen and exposure to high temperatures. In 2014, major duckweed losses occurred when air temperatures rose to 47°C with water temperatures of more than 40°C. Looking at the data from the Ecoferm model of 2015, we can see from the daily duckweed harvesting logs that in the second week of June the duckweed died because of high temperatures and a lack of oxygen. Because of the high temperatures, plants die and sink to the bottom. At the bottom, bacteria start to decompose the plants in which they use the oxygen available in the water. Toxic compounds like nitrite and sulphite will be formed, which will reduce the duckweed growth. The availability of oxygen used for the respiration of the duckweed will decrease and eventually more plants will die (also due to the high temperatures) (de Wilt, 2016). Due to thermodynamic principles, when temperature increases, less oxygen can be disolved in water. To prevent this, adiabatic cooling by atomization was realized at air temperatures above 37°C to lower the water temperature (to 35°C) while still keeping the irradiance as high as possible. In addition, oxygen was added by adding a submersible pump and aerators to prevent low dissolved oxygen levels in the water. At very high air temperatures (up to almost 40°C), plastic shields were installed that blocked direct incoming irradiance. A major disadvantage of this solution was that shading occurred, which influences the photosynthesis of the duckweed. However, to prevent the water temperature to rise above 35°C, it was a feasible solution. Finally, air coming from the calves stable was humidified in the bio-bed and blown into the greenhouse attic to bring in cool air and abduct the warm air (de Wilt, 2016).

After the oxygen problem due to the high temperatures, the dominant species in the pond was changed from Lemna minor to Spirodela polyrhiza. The main reason was that S. polyrhiza survives better in a low oxygen environment. The S. polyrhiza species has - besides different minimal, optimal and maximal levels to environmental growth influences - also some physical differences. When exhaustion of minerals occurs, the S. polyrhiza species will form turions. Turions are small, non-growing fronds. They are rootless and contain a high content of starch, which is an important energy source for the duckweed fronds (Landolt et al., 1987). The ABA (Abscisic acid) hormone will induce the turions (dormant bodies). Those turions are very tolerant to anaerobic conditions (Landolt et al., 1987). Not only low oxygen levels will cause the formation of these bodies. Other factors like low light intensity, high CO_2 concentration, low nitrogen levels and red light will also induce the formation (Newton et al., 1978). Due to the tolerance of anaerobic conditions, the S. polyrhiza species can withstand extremer temperatures than for example the L. minor species.

2.1.2. Temperature differences

In Figure 1 you can see that L. minor has a higher maximum growth rate than the S. polyrhiza species. This is a result of many temperature dependent chemical and physical processes like nutrient uptake, nutrient assimilation and transport, photosynthesis, respiration, diffusion of elements, water flow, permeability and many other enzymatic activity processes (Landolt et al., 1987). However, the temperature range of the high growth rate for L. minor is smaller than for S. polyrhiza. At around 32°C

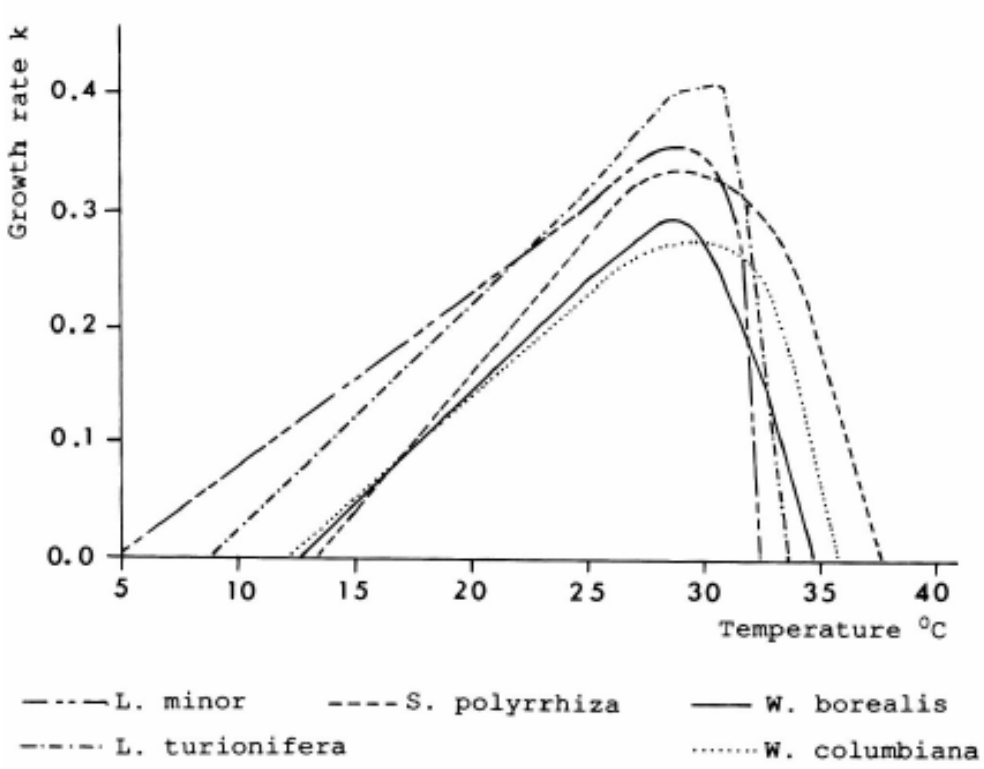

Figure 1: Growth rate of different Lemnaceae species in relation to temperature (Docauer (1983) as illustrated in Landolt et al. (1987))

the growth rate of L. minor will decrease very fast while S. polyrhiza starts decreasing at around 35°C. Optimal temperatures for photosynthesis and respiration are reached at 30 °C for S. polyrhiza according to Czopek (1967) and the figure Figure 1: Growth rate of different Lemnaceae species in relation to temperature (Docauer (1983) as illustrated in Landolt et al. (1987)). For L. minor, according to Ruigrok (2015), the optimal temperature is reached at 26 °C. For S. polyrhiza, the optimal temperature for photosynthesis is reached at 30°C (Czopek, 1967). The minimum temperature (where the growth rate is at least 10% of the maximum growth rate) for L. minor is 8°C according to (Landolt et al., 1987). For S. polyrhiza, the observed minimal temperature was at 13.5°C (Docauer (1983) as written in Landolt et al. (1987)). For short-term maximum temperatures, L. minor can tolerate temperatures between 40 and 60°C. At 42°C, the 50% lethality level (50% of the duckweed has died at this stage) is reached after 2 hours. At higher temperatures like 50°C, L. minor reaches the 50% lethality level already after 5 minutes. According to Ruigrok (2015), the Lemna species instantly dies at temperatures above 40.9°C and there is no growth at temperatures above 35.6°C. If we look at the data available about the duckweed cultivation at Ecoferm, we can confirm that when the water temperature becomes 35°C and higher, decease will be noticed. S. polyrhiza has a better adaptation to higher temperatures. It is able to withstand 24 hours at 50°C and a week at 40°C (Landolt et al., 1987). For long-term maximum temperatures, S. polyrhiza also has a higher temperature tolerance. As we can see, at temperatures up to 38°C, the Spirodela species still has a growth rate. However, at temperatures above 30°C, the respiration rate will increase, which will eventually result in a negative net assimilation rate, which causes decease. For the Lemna species, this point is almost the same. However, the curve decreases much faster and a maximum temperature of around 32°C can be observed (Landolt et al., 1987). Temperature values summarizing the values mentioned above are shown in Table 1.

Table 1: Temperature values for the species S. polyrhiza and L. minor

Species	Temperature values (°C)		
	minimum	optimal	maximum
S. polyrhiza	13.5	30	40
L. minor	5	26	35

2.1.3. Light intensity differences

Light saturation for photosynthesis depends on temperature. For low temperatures, the light saturation will be lower than at higher temperatures. The light saturation also differs between growth and photosynthesis rate. L. minor has a light saturation between 27 and 29°C that was observed at 17000 lux (17000x0.0185 = 314.5 $\mu mol/m^2s$ *) (Landolt et al., 1987). In the present model for Ecoferm by van den Top, the light saturation point was set to 342 $\mu mol/m^2s$ at a temperature of 27°C chosen by Lasfar et al. (2007). Light intensities below this point are ignored by the model Lasfar used. If we look at the figure below, optimal light intensities between 24°C and 29°C are observed at 9000 lux (9000x0.0185 = 166.5 $\mu mol/m^2s$). Nevertheless, the figure by Landolt et al. (1987) shows the light intensity in lux and Lasfar measured the energy content in photons. This means that it is difficult to compare the findings according to the influence of light intensity on growth rate because the light intensity in lux takes the whole light spectrum. The light intensity from photosynthetically active radiation (PAR) uses only a spectrum that is used by organisms that photosynthesize. In this report, the findings will be compared using the formula above to give an indication. In Figure 2 we can observe that indeed the highest growth rate for Lemna minor is found at about 29°C at 16000 lux and above.

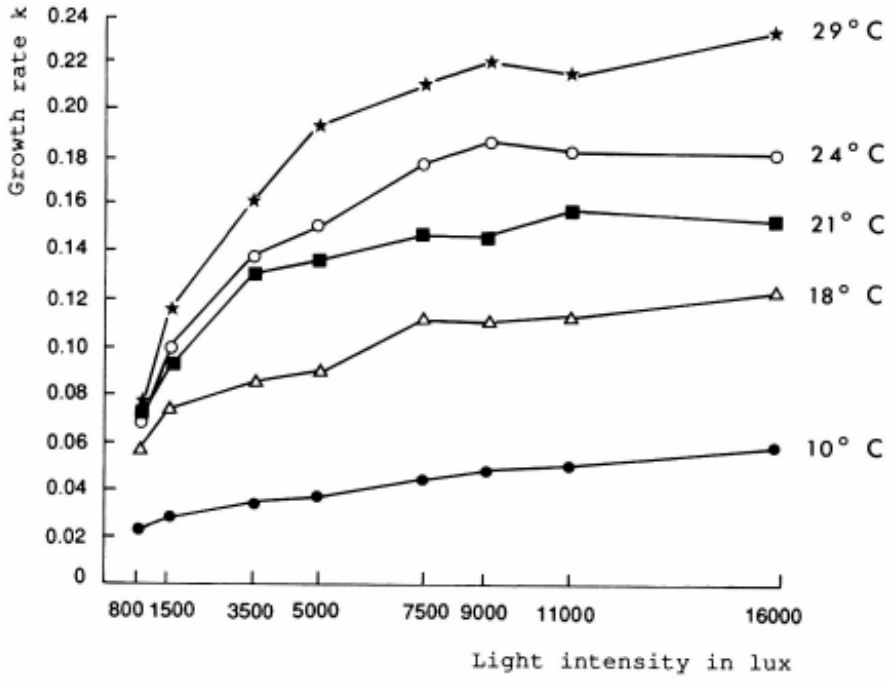

Figure 2: Growth rates of Lemna minor at different light intensities and temperatures (Ashby and Oxley (1935) as illustrated in Landolt et al. (1987))

* Conversion from lux to PAR found at: http://www.apogeeinstruments.co.uk/conversion-ppf-to-lux/

For S. polyrhiza, light saturation was found at 91 $\mathrm{J/m^2s}$ for normal fronds and 32 $\mathrm{J/m^2s}$ for turions by Czopek (1967). Converting these values into $\mu\mathrm{mol/m^2s}$ gives:

$$Light\ Saturation\ normal\ fronds = I_{joules} * \ \xi = 91 * 4.59 = 417.7\ \mu\mathrm{mol/m^2s} \quad [1]$$

$$Light\ Saturation\ turions = I_{joules} * \ \xi = 32 * 4.59 = 146.9\ \mu\mathrm{mol/m^2s} \quad [2]$$

where I_{joules} is the light intensity in Joules and ξ is the conversion factor from Joules to photons. Gaponenko and Staxhetskii (1969) found a light saturation of 92 $\mu\mathrm{mol/m^2s}$, which is quite low. From Figure 3 the saturation point is near 200 $\mu\mathrm{mol/m^2s}$ for S. polyrhiza and near 350 $\mu\mathrm{mol/m^2s}$ for L. minor. This means that S. polyrhiza will reach a steady state light intensity for photosynthesis earlier than the L. minor species and will be more productive (concerning light intensity) at cloudy environments.

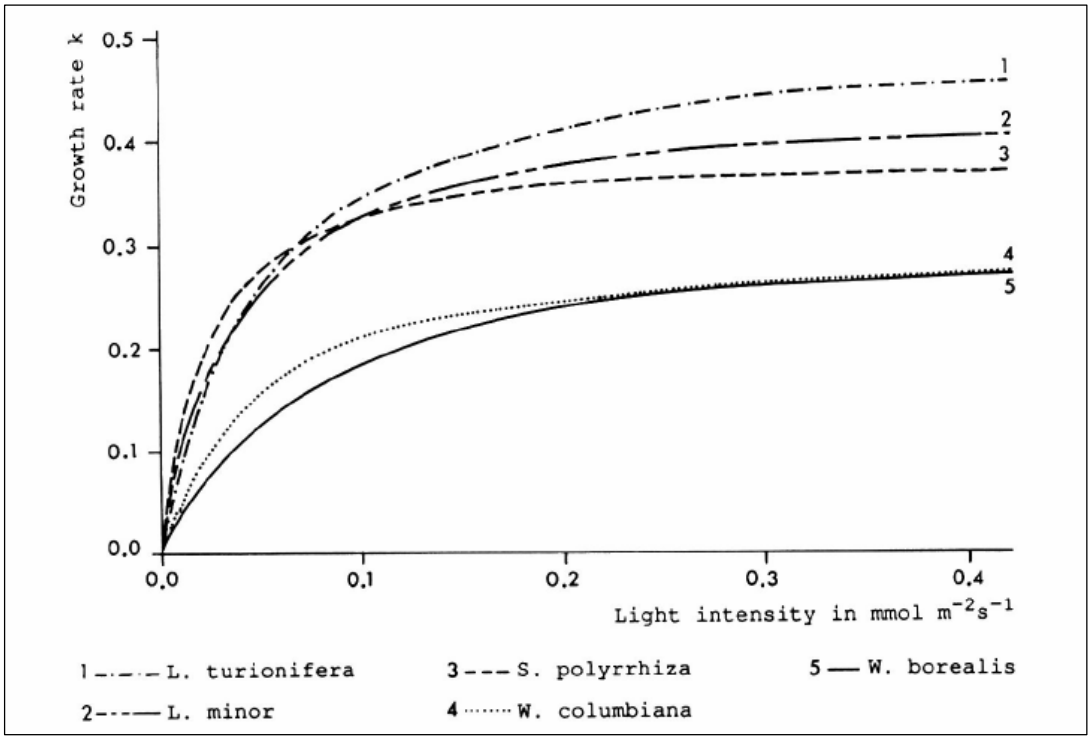

Figure 3: Growth rate of different species in relation to light intensity (Docauer (1983) as illustrated in Landolt et al. (1987))

2.1.4. pH

Different pH's in the water influences the nutrient uptake. Especially the nutrients phosphor and nitrogen are important for Lemnaceae. The optimal pH values differ between species. Duckweed species have a wide range of pH tolerance. Literature observed pH ranges from 3 to 10 for different species. For S. polyrhiza, the optimal growth rate needs a pH value between 6.5 and 7.5 (Islam and Khondker, 1991). The minimal pH value for the Spirodela species is 3.7 and the maximum is 9.0. For Lemna minor, the optimal pH value lies between 6.2 and 6.7 (Landolt et al., 1987). McLay (1976) observed an optimal value of 6.2. The Lemna species is somewhat more tolerant to a lower pH, namely 3.0. This species can also withstand higher pH values up to 10.5 (Landolt et al., 1987). Table 2 shows an overview of the values mentioned above.

Table 2: pH values for the species S. polyrhiza and L. minor

Species	pH values		
	minimum	optimal	maximum
S. polyrhiza	3.7	6.5 - 7.5	9.0
L. minor	3.0	6.2 - 6.7	10.5

2.1.5. Nutrients

The main nutrients that influence the growth of duckweed are nitrogen and phosphorus. The main source of nitrogen preferred by duckweeds is ammonia nitrogen (NH_4-N) as source of nitrogen. Under normal conditions, urea is a good fertilizer for duckweed because it will be rapidly converted to ammonia under normal conditions (Hasan and Chakrabarti, 2009). This is also the case at the Ecoferm project where urea from the calves is used for the growth of the duckweed. At low nitrogen levels, duckweed species prefer ammonium instead of nitrate because of the uptake efficiency. Ammonium can pass the plants membranes easier than nitrate. Nitrate has to be assimilated first.

Lasfar used a Michaelis-Menten equation to determine the intrinsic growth rate as a function of

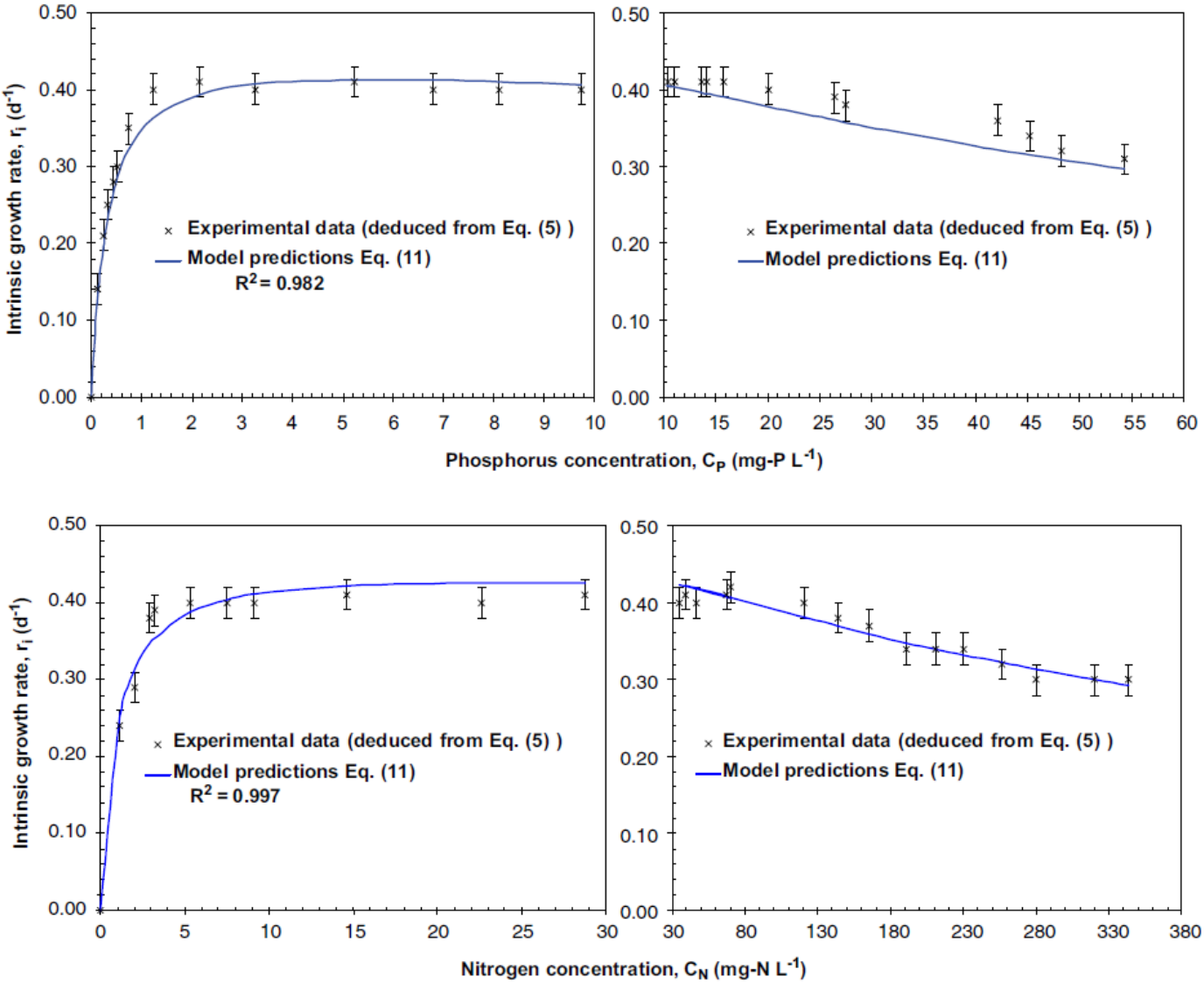

Figure 4: Intrinsic growth rate in dependence of nitrogen and phosphorus concentration; the bars represent the maximum error deduced from the nitrogen and phosphorus mass balances (Lasfar et al., 2007).

nitrogen and phosphorus concentration. Figure 4 below describes that at concentrations between 10 and 15 $\mathrm{mg-N/L}$; L. minor is saturated and reaches its highest intrinsic growth rate. At concentrations above 30 $\mathrm{mg-N/L}$, growth will be inhibited. For phosphorus, saturation occurs at around 3 $\mathrm{mg-P/L}$ and growth will be inhibited from 10 $\mathrm{mg-P/L}$ and higher. After nitrogen, phosphorus is the second major limiting nutrient and it is essential for rapid growth.

Nutrient requirements for different species are described in Table 3:

Table 3: Supply requirements of phosphorus and nitrogen for S. polyrhiza and L. minor with findings from several papers: (1) Lüönd (1983), (2) Docauer (1983), (3) Eyster (1966), (4) Aebli (1986), (5) Müller (1983) (modified table from Landolt et al. (1987))

Species	Supply requirement P and N in mM					
	min. N	min. P	opt. N	opt. P	max. N	max. P
S. polyrhiza	0.04 (1)	0.00046 (2)	5.0 - 30.0 (3)	0.1 - 1.0 (3)	120 (3)	7.75 (1)
	0.006 (2)	0.0028* (1)	1.0 - 25.0 (1)	0.014 - 0.35 (1)	100 – 120 (5)	10 (2)

L. minor	0.005 (2) 0.008 (1)	0.00045 (2) 0.00011 (1)	0.2 - 25.0 (1)	0.014 - 0.35 (1) 0.01 - 0.2 (4)	100 – 150 (5)	1.75 (1)

*forming turions at lower concentrations

Effects of low nutrient availability are described in Table 4:

Table 4: Effects on low nutrient availability: - is decreasing effect, + is increasing effect, 0 = no effect, S = S. polyrhiza species, L = L. minor species (modified table from Landolt et al. (1987))

Characteristics	N effect	Species	P effect	Species
Area of frond	-	S + L	-	S + L
Dry weight	-	S	-	S + L
Length/width ratio	0	S + L	0	S + L
Length of root	+	S + L	+	S + L
Length of root cells	+	L	+	L
Turion formation	+	S	+	S
Starch content	-	L		
Anthocyanin content	+	S	+	S
Protein content			0	L
Chlorophyll content	-	L		
Content of free amino acids	-	L		
Oxalate content			-	L
Respiration rate	-	L	-	L
Glycolysis rate	-	L		
Photosynthesis rate			-	L
Phosphatase activity			+	L

From these two tables it is clear that the different species require different amounts of nutrients. However, the optimal N and P requirement values are in the same range for both species. This means that it is possible to breed two species in one pond (which is the case now at Ecoferm).
Table 4 shows the effects of low nutrient availability. As already mentioned, in critical situations, S. polyrhiza will form turions. Also growth in root length will be induced by both species. Concerning the nutrient uptake from Table 4, it is clear that L. minor is more affected by low nutrient availability than S. polyrhiza.

2.1.6. Fronds

Duckweed species in general do not have any true leaves or stems, the leaf-like bodies are called fronds or thalli.
L. minor is also known as 'lesser duckweed' because it has small fronds. In contrary, S. polyrhiza, also known as 'giant duckweed' is the largest duckweed species known. L. minor has circular to oval fronds with a diameter of 2 to 5 mm. It occurs as a single plant and can develop up to five fronds that are connected (this is also the case for S. polyrhiza). The Lemna species has one root on every frond; this is in contrary with the Spirodela species that has clusters with 4 to 16 roots for each frond.

S. polyrhiza has fronds that have a diameter of 4 to 10 mm. Both species have the ability to flower, however this rarely happens. Both species can be found in slow moving fresh waters like ponds or standing rivers. Where Lemna is a very dominant species which forms a mat on the water surface that blocks incoming light for other water life, S. polyrhiza often grows between other duckweed species (like L. minor). This makes it possible for the species to withstand competition from underlying macrophytes that also use nutrients from the water. Those macrophytes will not be able to receive sunlight, because the Lemna canopy blocks it. Eventually the macrophytes will not survive (from the free floating plants database of the Department of Ecology State of Washington). In Figure 5, the two species are drawn:

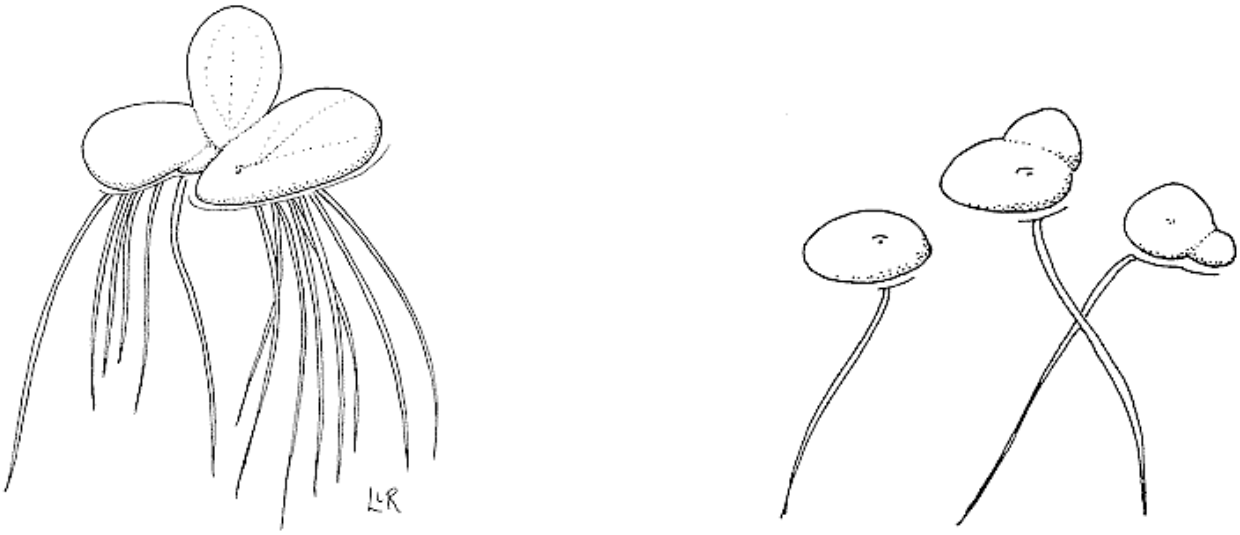

Figure 5: Drawings of S. polyrhiza on the right and L. minor on the left

2.2. Photosynthesis

2.2.1. Reactions

Light plays a major role in the photosynthesis in a leaf. Leaves of the plant can convert light energy from wavelengths of 400 to 700 nm to chemical energy which the plant can use for its growth. The light energy will be absorbed by the chloroplasts in a leaf. In these chloroplasts several reactions occur during the photosynthesis to create this chemical energy. A by-product that is created in the photosynthesis is oxygen.
During photosynthesis, two reactions take place: a light and a dark reaction.

a. Photolysis (light reaction):

$$2\,H_2O \rightarrow O_2 + 4\,H^+ + 4\,e \qquad [3]$$

b. CO_2-reduction (dark reaction):

$$CO_2 + ATP + NADPH \rightarrow Sugar\ (Glucose\ and\ other\ organic\ compounds) \qquad [4]$$

This leads to one general photosynthesis reaction:

$$CO_2 + H_2O + Light\ energy \rightarrow Glucose + O_2 \quad [5]$$

A chloroplast contains thylakoids and stroma. The light-dependent reactions occur in the thylakoids and the light-independent reactions will occur in the stroma (for C_3-plants).

2.2.2. Light reactions (Raven et al., 2005).

Photosystem II:
Light reactions occur in grana, stacks of thylakoids. The thylakoid membranes contain clusters of pigments. The pigments are chlorophyll a, chlorophyll b and carotenoids. Those pigments absorb the photons from the incoming light with their so-called antennas. When a photon is absorbed, electrons coming from the water splitting (photolysis) complex where 2 H_2O is divided in 4 H^+, O_2 and 4 e^- electrons. These electrons will gain a boost and are then transferred to a higher energy level. The transferred electron will be accepted by an electron acceptor called Pheophytin via a special molecule called P680 (electron donor). Via several redox reactions, the electrons will be transferred to a Cytochrome b_6/f complex. In this complex, the electron transport is responsible for creating an H^+ gradient, which is used to produce ATP that can be used in the Calvin cycle later in the process. Then the electrons are transferred to photosystem I.

Photosystem I:
In photosystem I, the electrons will undergo a second transfer to a higher energy level by a light reaction. A molecule called P700 acts as an electron donor. The electrons will be transferred to 5 electron acceptors. Eventually, an enzyme will transfer the electrons to the catalyser $NADP^+$ that forms NADPH which can be used in the Calvin cycle.

Figure 6 gives an overview of the processes.

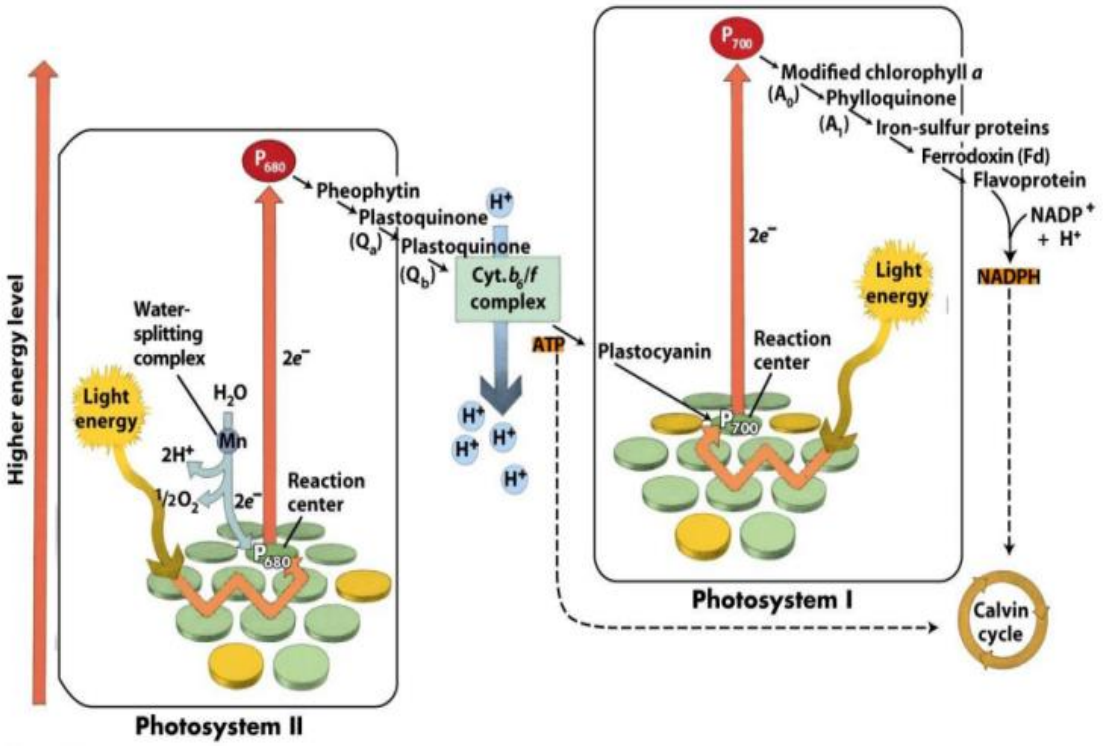

Figure 6: Light reactions with Photosystems I and II (Raven et al., 2005)

The reactions that occur in light reactions are described below.

1. Cytochrome b_6/f complex

$$ADP + P_i\ (inorganic\ phosphate) + light\ energy \rightarrow ATP \quad [6]$$

2. Photosystem I

$$NADP^+ + H^+ + 2\,e^- \rightarrow NADPH \quad [7]$$

3. Water splitting complex

$$2\,H_2O \rightarrow O_2 + 4\,H^+ + 4\,e^- \quad [8]$$

2.2.3. Dark reaction (Calvin cycle)

The Calvin cycle is responsible for the carbon metabolism in plants located in the stroma. The light reactions described above deliver the needed energy in the form of ATP and NADPH. Eventually Glucose ($C_6H_{12}O_6$) will be formed (Fridlyand and Scheibe, 1999).
During a cycle, the following reactions take place:

1. $3\,C_5H_8O_{11}P_2 + 3\,CO_2 + 3\,H_2O \rightarrow 6\,C_3H_5O_7P$ (Catalysed by Ribulose-1,5-bisphosphate (RuBP) enzyme) [9]
2. $6\,C_3H_5O_7P + 6\,ATP \rightarrow 6\,C_3H_4O_{10}P_2 + 6\,ADP + 6\,H_2O$ [10]
3. $6\,C_3H_4O_{10}P_2 + 6\,NADPH + 6\,H_2O + 6\,H^+ \rightarrow 6\,C_3H_5O_6P + 6\,NADP^+ + 6\,P_i + 6\,H_2O$ (takes place 6 times. 6 Glyceraldehyde 3-phosphate (G3P) is needed: 5 G3P will be used for continuing the cycle, 1 will be used for sugar synthesis) [11]
4. $5\,C_3H_5O_6P + 2\,H_2O \rightarrow 3\,C_5H_9O_8P + 2\,P_i$ [12]
5. $3\,C_5H_9O_8P + 3\,ATP \rightarrow 3\,C_5H_8O_{11}P_2 + 3\,ADP + 3\,H_2O$ [13]

In general:

$$3\,CO_2 + 6\,NADPH + 5\,H_2O + 9\,ATP \rightarrow glyceraldehyde-3-phosphate\ (G3P) + 2\,H^+ + 6\,NADP^+ + 9\,ADP + 8\,P_i \quad [14]$$

The sixth $G3P$ molecule is used for the synthesis of glucose and fructose. In daylight, the triose will be used to make sugars (sucrose) for storage and transport. In the night, the triose is converted to starch, which will be used for the synthesis of glucose and maltose. The two sugars are used to form sucrose which can again be used for growth and storage (Raven et al., 2005). The processes described above are shown in an overview in Figure 7.

2.2.4. Non-photochemical quenching

To protect Photosystem-II to a very high amount of incoming photons, non-photochemical quenching (NPQ) or fluorescence takes place when excessive energy (photons) is absorbed. In a dynamic environment with fluctuating irradiance, it is an important process. Not only fluctuating irradiance affects quenching, also changes in temperature are important in this process. When excessive energy is available, NPQ makes it possible to emit the observed photons as heat and return the state back to the ground level (PSII). This process is called fluorescence. When light saturation is reached, the NPQ processes will be executed and chloroplasts and thylakoids will be protected (Kaiser et al., 2015).

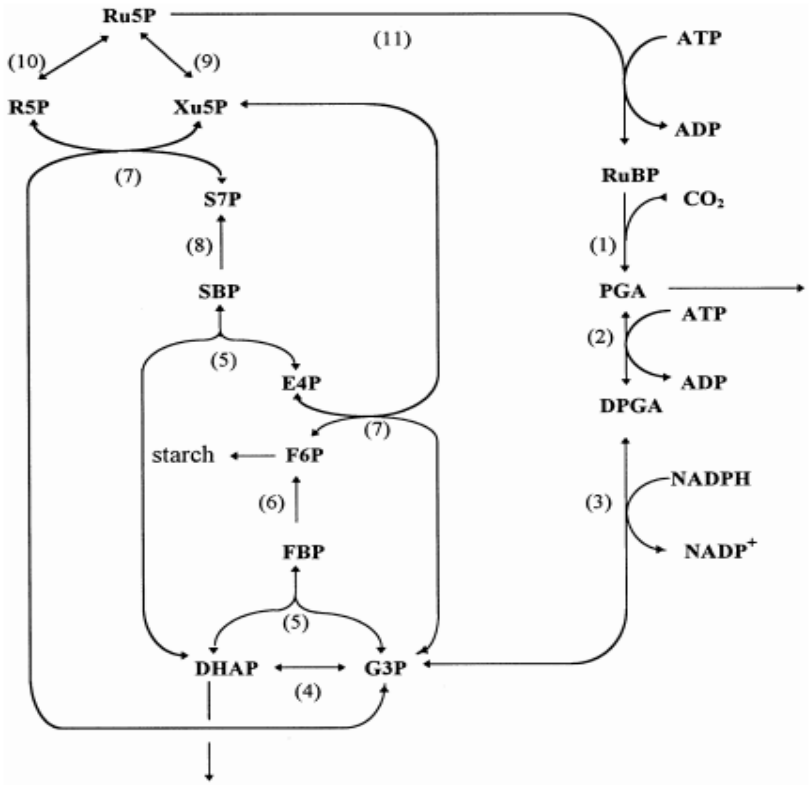

Figure 7: Calvin cycle described. DHAP, dihydroxyacetone phosphate; DPGA, 1,3-biphosphoglycerate; E4P, erythrose 4-phosphate; F6P, fructose 6-phosphate; FBP, fructose 1,6-biphosphate; G3P, clyceraldehyde 3-phosphate; PGA, 3-phosphoglycerate; RuBP, ribulose 1,5-biphosphate; Ru5P, ribulose 5-phosphate; R5P, ribose 5-phosphate; SBP, sedoheptulose 1,7-biphosphate; S7P, sedoheptulose 7-phosphate; Xu5P, xylulose 5-phosphate. The corresponding enzymes are indicated by numbers, (1) Rubisco; (2) 3-phosphoglycerate kinase; (3) NADP-GAPDH; (4) triosephosphate isomerase; (5) aldolase; (6) FBPase; (7) transketolase; (8) sedoheptulose 1,7-biphosphate; (9) ribulose 5-phosphate 3-epimerase; (10) ribose 5-phosphate isomerase; (11) PRK. (Fridlyand and Scheibe, 1999)

2.2.5. CO_2 diffusion

CO_2 has a low diffusion coefficient in water. This means that a high CO_2 concentration is needed for the carboxylation to maintain the same use of CO_2 for terrestrial plants. Aquatic plants have high saturation rates for CO_2. The Rubisco (the enzyme that carboxylates the CO_2) kinetics is more similar to C4 plants than to C3 plants (Raven et al., 1985). This means that in contrary to terrestrial plants, a higher CO_2 concentration is needed for CO_2 fixation at carboxylation sites.
Before CO_2 assimilation can take place, CO_2 has to diffuse from the surrounding medium to the carboxylation sites within the chloroplasts. During this pathway, CO_2 faces a series of resistances from sub stomatal cavities to the chloroplasts. At the sites of carboxylation, Rubisco consumes the absorbed CO_2 and performs the CO_2 fixation catalyses the carboxylation whereby sugars can be formed (Evans et al., 1994).
In a leaf there are three important barriers that limit the CO_2 diffusion from outside the leaf to the Rubisco enzyme: boundary layer, stomata and mesophyll. To model these resistances, Fick's law can be used with influences from the partial pressure of CO_2 (Hikosaka et al., 2015). Stomatal resistance is highly influenced by environmental factors such as wind and temperature differences. The total mesophyll resistance is determined by an intercellular airspace resistance and several liquid resistances.
The total mesophyll resistance is separated into two phases, namely the gaseous and the liquid phase. Given in series:

$$r_m = r_{ias} + r_{liq} \quad [15]$$

where r_m is the total mesophyll resistance computed from the intercellular air resistance (r_{ias}) and several liquid resistances(r_{liq}). When CO_2 has passed the intercellular air spaces, it enters the liquid phase. First CO_2 dissolves in the water-filled pores of the cell wall and then diffuses through the plasma membranes. Before it can diffuse to the chloroplast where it can reach the Rubisco enzyme, the CO_2 has to pass the cytosol, the chloroplast envelope and finally before it enters the chloroplast, the stroma. While the CO_2 passes the several stages after each other, the total liquid mesophyll resistance can be seen as a sum of series (Evans et al., 2009):

$$r_{liq} = r_{wall} + r_{plasma\ membrane} + r_{cytosol} + r_{chloroplast\ envelope} + r_{stroma} \quad [16]$$

Van Ooteghem (2007) approaches the resistances as in Bot (1983) and describes the process based on the resistances from the H_2O diffusion. The model used in this thesis will also be described in the way Ooteghem did**.** Bot (1983) describes the diffusion as the pathway from the leaf surface to the inside based on the morphology of the leaf. The CO_2 has to penetrate the cuticula and the stomata after it diffuses through the boundary layer and then has to meet the resistance by the cavity. These resistances can be described as follows:

$$R_{CO_2} = \frac{R_{cut} * R_s}{R_{cut} + R_s} + R_{carboxylation} \quad [17]$$

The first part of the equation can be compared with the way Bot described the water vapour transfer in Figure 8:

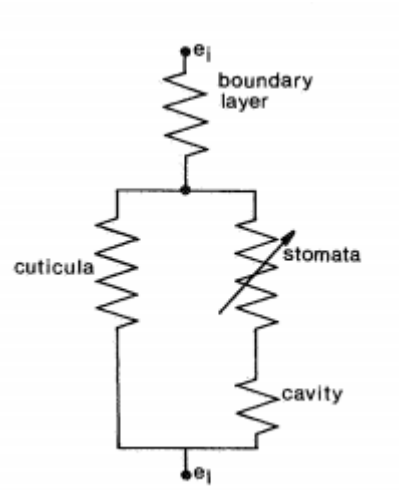

Figure 8: Resistances to water vapour transfer (Bot, 1983)

2.2.6. Stomatal conductance

With the presence of stomata, gaseous exchange can be possible. The stomata can be closed or opened due to the turgor state of the guard cells parallel on both sides of the stomatal cells. For Lemnaceae, the stomatal cells are located on the upper side of the leaves. For the Lemna species, the cells are not moveable and remain open no matter what condition is met (Landolt et al., 1987). Transpiration in Lemna proceeds in parallel evaporation described above. Due to the lack of cytoplasmic structures and organelles, the activity of the stomatal cells is absent or scarce. The Lemna species has a thick ventral (guard) cell wall that makes any movement impossible (Severi and Fornasiero, 1983).

2.2.7. Respiration

When there is not enough light available (for example during the night), photosynthesis cannot take place because electrons are not able to transfer to a higher energy level due to the lack of incoming photons.
Respiration is defined as the sum of all metabolisms. This means that the plant uses O_2 and produces CO_2, which is often seen as the breathing of a plant. This means a loss of organic carbon, which influences the productivity in the plant. Respiration is an important process though, because otherwise a plant will not survive. The process of respiration will occur at daylight as well as at night. During the day, both (photo)respiration and photosynthesis will take place and during the night, only respiration will take place (dark respiration). For this thesis, respiration will be seen as one constant with same values for day and night.
As mentioned by Filbin and Hough (1985), photorespiration rates in submersed plants are somewhat lower than terrestrial plants under the same climate. This may be due to the less extreme conditions to which the submersed plants are exposed. High water availability leads to less need for stomatal closure or maintenance of rapid atmospheric gas exchange. This could also minimize the photorespiration. Productivity influences of Lemna minor were most strongly due to light intensity and temperature.
Filbin and Hough also stated that the diurnal progression in the light correlated inversely with the net photosynthesis and positively with the environmental parameters known to enhance the photorespiration. For Lemna minor, the respiration rates seemed to be relatively low.
As the Light:Dark ratios on several other researches on submersed angiosperms, the respiration rates appear to be below unity for Lemna minor. Due to the high temperature optimum and high light saturation capacity found by Filbin and Hough, Lemna minor showed characteristics for a C_4 plant. Also the low CO_2 compensation point is a characteristic for a C_4 plant. However, Wedge and Burris (1982) found that Lemna minor, as other duckweed species, is a C_3 plant, which will be discussed below. This means that the low rates of photorespiration in Lemna minor is related to other factors than to C_4 biochemistry characteristics. What can play a huge role in the low respiration rates is the ready access to water. Because there is little need for stomatal closure in high temperature and high light, it means that there is a continue gas exchange with minimal internal O_2 build-up as well as minimal CO_2 limitation (Filbin and Hough, 1985). Especially when there is CO_2 added to the system, which is done in the Ecoferm project. Filbin and Hough conclude that the major limiting factor would be the inorganic carbon content of the water.

2.2.8. C4 or C3

C_4 plants have different photosynthetic responses to light intensity than C_3 plants. Where C_3 plants need $1/3$ to $1/2$ of full sunlight for saturation and C_4 plants need more than full sunlight for photosynthetic saturation. Also the temperature tolerance is different between C_3 and C_4 plants, C_4 plants can withstand higher temperatures and have a higher optimal growth temperature than an average C_3 plant. Wedge and Burris found light saturation for Lemna and Spirodela plants from 600 to 1200 $\mu mol/m^2s$. This is higher than $1/3$ to $1/2$ of full sunlight but lower than full sunlight (1400 $\mu mol/m^2s$). However, Wedge and Burris observed that for younger Lemna fronds, photo inhibition will occur at light intensity above 1200 $\mu mol/m^2s$. This indicates that duckweed fronds are closer to C_3 than C_4 plants (C_4 plants are not inhibited at light intensities that are lower than full sunlight). Also, as Bauer et al. (1976) (as written in Wedge and Burris (1982)) observed, the photosynthetic ^{14}C-labeling is typical to a C_3 plant. The temperature response on the other hand is different from a typical C_3 plant. Duckweeds have an optimal temperature between 25-30°C, for C_3 plants this is around 20°C. However a change in photosynthetic rate was less existing than it was the case with C_4 plants (Wedge and Burris, 1982). This proves that duckweed species can be seen as a C_3 plant.

2.3. Diffuse lighting

Most of the time, horticultural plants grow in layers from top to bottom. While plants in Dutch greenhouses have a high leaf area index, a large quantity of the incoming direct light is absorbed by the upper layer. This means that a middle and bottom layer receive less light and produce much less due to a lack of photosynthesis (Hemming et al., 2008). Diffuse light, which can be realized by special greenhouse glazing, can penetrate into the canopy because the light is scattered by molecules in the atmosphere and enters the canopy from different directions. This means that diffuse light can be used more efficiently and leads to a higher photosynthetic rates (Li et al., 2014). Li also noticed that a more homogeneous distribution of photon flux density (or incoming light) was the most important factor leading to a higher leaf photosynthetic capacity. Apart from this, diffuse lighting makes it possible to allow more light into the greenhouse and prevent shading from upper layers in the canopy. Furthermore, diffuse light can be advantageous in contrast to direct lighting considering solar position and seasonal light conditions where direct light irradiates the canopy in different angels throughout the day and the season and diffuse light is available from different angles (Li and Yang, 2015). Falge et al. (2002) noticed that due to diffuse lighting, the tendency to induce canopy photosynthetic saturation is less in contrast to direct lighting and has a higher light use efficiency increasing with the level of radiation. Also temperatures as well as vapour pressure deficit in the greenhouse are lower when the horticulturist realises diffuse lighting as much as possible.
However, the pond used in at the Ecoferm farm does not have different terrestrial layers where light has to penetrate to. It is assumed that the duckweed canopy forms one layer over the whole pond. This can be realized by a constant circulation made by pumps in the pond (de Wilt, 2016). While light does not have to penetrate the layer, it does not matter in what amount light is absorbed by the canopy. Because eventually the photons in the light are used for the photosynthesis and there is no difference in photons of direct and diffuse light. Because diffuse light can penetrate through terrestrial layers, it can be advantageous to make use of more diffuse light instead of direct lighting. While this is not the case at the Ecoferm greenhouse, it is not needed to research the advantage of diffuse in contrast to direct lighting because it is likely possible to observe no to almost none photosynthetic improvements (Slegers, 2016).

3. Current model

The current growth rate model is determined by equations from Lasfar et al. (2007), McLay (1976) and Frédéric et al. (2006). The model determining intrinsic growth rate includes functions for the growth depending factors temperature, photoperiod, phosphorus and nitrogen concentrations and pH grade. Also a maximum intrinsic growth rate constant (R) (depending on maximum growth factors (above mentioned)) is added to limit the modelled growth to a maximum (lowest deviation between model and experimental data (van den Top, 2014). The specific growth rate depends on mat density and cultivation (retention) time.

The intrinsic growth rate (1/d) can be expressed as follows:

$$r_i(T, C_P, C_N, E) = R * f(T) * g(C_p) * h(C_n) * s(E) * j(pH) \qquad [1/d] \qquad [18]$$

where $f(T)$, $g(C_p)$, $h(C_n)$, $s(E)$ and $j(pH)$ are functions for the already mentioned growth factors.

3.1. Growth factors

3.1.1. Phosphorus and nitrogen

The growth factor for phosphorus and nitrogen (1/d) is based on Michaelis-Menten kinetics; see also Figure 4 mentioned in paragraph 2.1.5. This means that the growth of duckweed in accordance to P and N concentrations will be determined by saturation and inhibition rates. This can be described by the following equation:

$$r_{i(P,N)} = \alpha_{P,N} * \frac{C_P}{(C_P + K_P)} * \frac{K_{IP}}{(K_{IP} + C_P)} * \frac{C_N}{(C_N + K_N)} * \frac{K_{IN}}{(K_{IN} + C_N)} \qquad [1/d] \qquad [19]$$

where $\alpha_{P,N}$ is a constant for the maximum intrinsic growth rate at a certain temperature, photoperiod and P and N concentrations. C_P and C_N are the concentrations for phosphorus and nitrogen respectively and K_P, K_N, K_{IP}, K_{IN} are constants for saturation and inhibition of phosphorus (P) and nitrogen (N).

3.1.2. Temperature

The growth factor for temperature effects (1/d) is based on Figure 9 at the next page and a modified van 't Hoff-Arrhenius relationship, expressed as follows (Lasfar et al., 2007):

$$r_{i(T)} = \alpha_T * \theta_1^{\left[(T-T_{op})/T_{op}\right]^2} * \theta_2^{(T-T_{op})/T_{op}} \qquad [1/d] \qquad [20]$$

where α_T is again a constant for the maximum intrinsic growth rate (which is obtained at $T = T_{op} = 26$°C in this case). θ_1 and θ_2 are two non-dimensional constants smaller than 1. T and T_{op} are the observed temperature and the optimal temperature respectively.

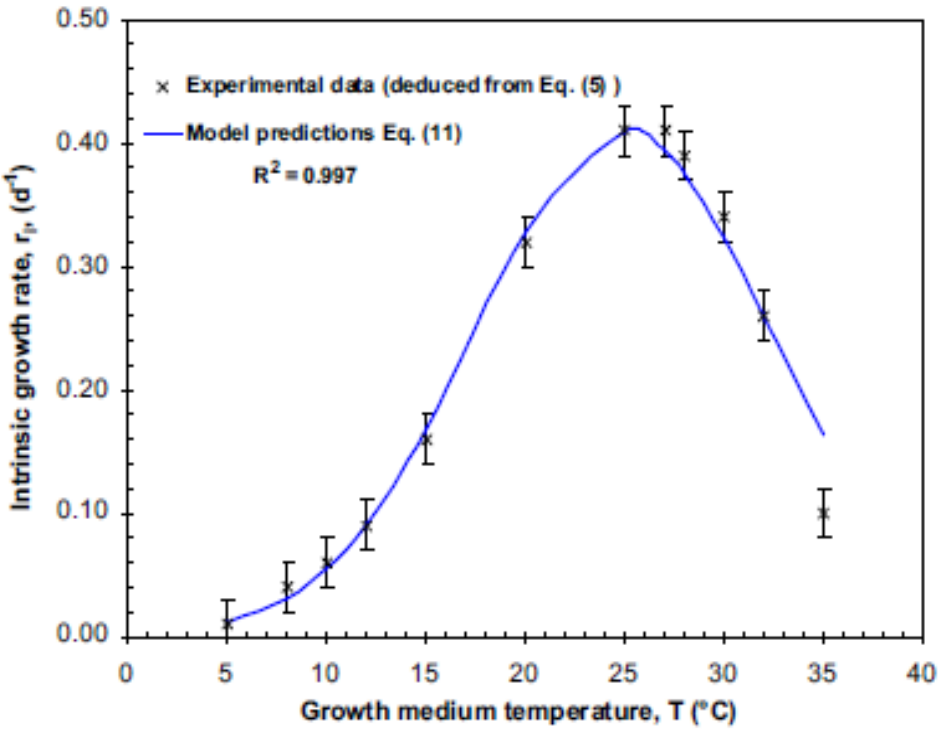

Figure 9: Intrinsic growth rate in dependence of temperature; the bars represent the maximum error deduced from the nitrogen and phosphorus mass balances (Lasfar et al., 2007)

3.1.3. Photoperiod

The photoperiod is determined as follows: when a specific saturation rate for photosynthesis for Lemna minor is reached (342 µmol/m^{2}s), then light intensity is not a limiting factor for the growth of duckweed (L. minor). Within an average of 10 minutes, the limitation is determined and when an average light intensity above the saturation rate is observed, the limitation is set to 1 (not limiting). For one day, these values are averaged over one hour. A minimum value of 2 hours per day will be added at the end (when in one day the observed photoperiod is 0, a minimum value of 2 will be obtained) (van den Top, 2014b).

The photoperiod, as calculated in van den Top (2014b) will be as follows:

$$E = \frac{\sum l_I}{6} + E_{min} \qquad [h] \qquad [21]$$

where l_I is the limitation factor (0 or 1) and E_{min} the minimum photoperiod.

The growth rate for L. minor depending on photoperiod (1/d) can be calculated with the following equation (Lasfar et al., 2007):

$$r_{i(E)} = \alpha_E * \theta_3^{\left[\frac{E-E_{op}}{E_{op}}\right]^2} * \theta_2^{\frac{E-E_{op}}{E_{op}}} \qquad [1/d] \qquad [22]$$

where α_E is the maximum obtained growth rate (at $E = E_{op} = 13h$) from experimental results, (which can be read from Figure 10) and θ_3 and θ_4 are two non-dimensional constants smaller than 1. This equation also follows a modified van 't Hoff-Arrhenius relationship.

3.1.4. pH

For the determination of the pH limitation, a 5th degree polynomial model by McLay (1976) is used with an optimal value of 6.2. The model used by McLay to calculate the growth dependence on pH is as follows:

$$g = -1.24851 + 0.662p - 0.08514p^2 - 0.00052p^3 + 0.0008p^4 - 0.00004p^5 \qquad [1/d] \qquad [23]$$

where p is the observed pH value.

The growth model from Lasfar et al. (2007) does not take a growth factor for pH into account. Van den Top (2014) used the maximum growth rates obtained by Lasfar et al. (2007) and McLay (1976) - 0.45 (1/d) and 0.27 (1/d) respectively - and expressed them as a percentage where 100% means optimal growth.

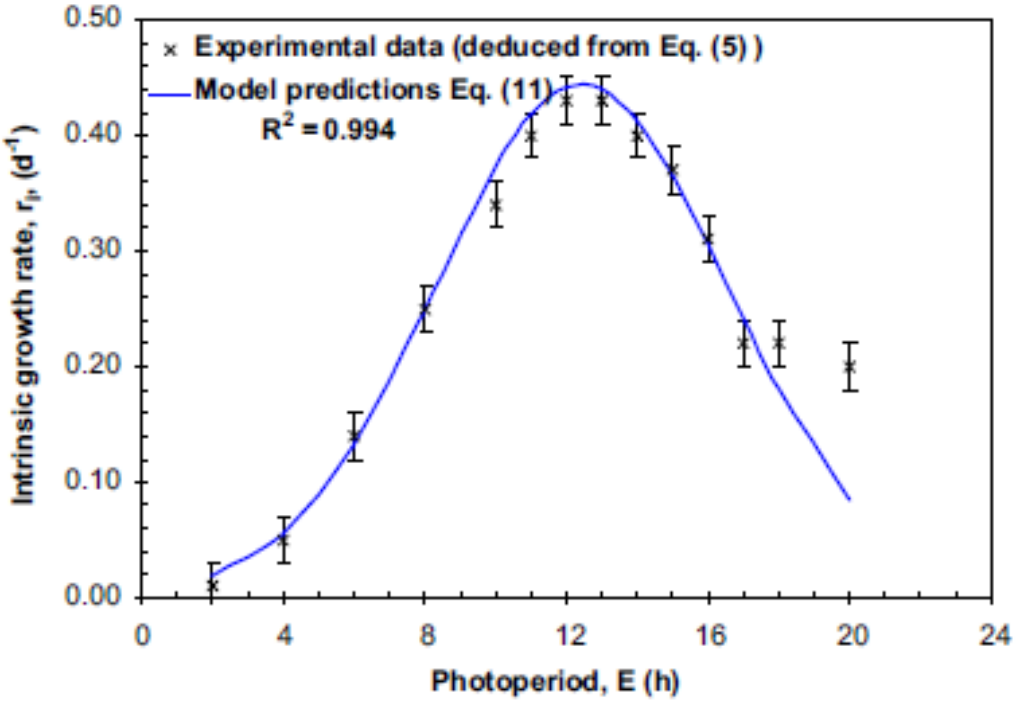

Figure 10: Intrinsic growth rate in dependence of photoperiod; the bars represent the maximum error deduced from the nitrogen and phosphorus mass balances (Lasfar et al., 2007)

The intrinsic growth rate for pH (1/d) is expressed as follows (van den Top, 2014):

$$r_{i(pH)} = r_{i,max} * \left(\frac{g}{g_{max}}\right) \qquad [1/d] \qquad [24]$$

where $r_{i,max}$ is a constant for the maximum intrinsic growth rate determined by Lasfar et al. (2007) and g_{max} is the maximum intrinsic growth rate determined by (McLay, 1976).

3.2. Global model

3.2.1. Intrinsic growth rate

Eventually, when combining the different growth factors (temperature, photoperiod, nutrients (P and N) and pH) with each other, the following equation for the intrinsic growth rate of duckweed follows:

$$r_i = R * \theta_1^{\left[(T-T_{op})/T_{op}\right]^2} * \theta_2^{(T-T_{op})/T_{op}} * \theta_3^{\left[\frac{E-E_{op}}{E_{op}}\right]^2} * \theta_2^{\frac{E-E_{op}}{E_{op}}} * \frac{C_P}{(C_P + K_P)} * \frac{K_{IP}}{(K_{IP} + C_P)} * \frac{C_N}{(C_N + K_N)} * \frac{K_{IN}}{(K_{IN} + C_N)} * \left(\frac{g}{g_{\max}}\right) \quad [1/d] \quad [25]$$

3.2.2. Specific growth rate

The specific growth rate (1/d) was determined with equations from Frédéric et al. (2006) and depends on the intrinsic growth rate mentioned above, the mat density and the retention time (which is the time between two harvesting moments).

The equation for the specific growth rate (1/d) in van den Top (2014) is as follows:

$$r_s = \frac{1}{t} * \ln\left(\frac{D_L}{(D_L - D_{0)}}\right) * e^{-r_i * t} + D_0) \quad [1/d] \quad [26]$$

where D_L and D_0 are parameters determining the mat density described below, r_i is the intrinsic growth rate, described above and t is the retention time (time between two harvesting moments).

Mat density

The mat density that describes the density of the duckweed area was determined by the integrating the mat density over the time (Lasfar et al., 2007).

$$\frac{dD}{dt} = \frac{(D_L - D)}{D_L} * r_i * D \quad [mg/m^2 s] \quad [27]$$

where D is the mat density at time t.

After integration, the mat density can be found with the following equation:

$$D = \frac{D_L * D_0}{(D_L - D_{0)} * e^{-r_i * t} + D_0} \quad [mg/m^2 s] \quad [28]$$

where $(D_L - D_{0)}$ is the produced duckweed with initial mat density D_0 after time t.

At the next page, Table 5 gives an overview of the parameters used in this model.

Table 5: Overview of the parameters used to determine the current model used by van den Top (2014).

Name	Value	Unit	Content
C_N	Input data	$\mathrm{mg-P/L}$	N concentration
C_P	Input data	$\mathrm{mg-P/L}$	P concentration
D	Eq.	$\mathrm{g/m^2 s}$	mat density at time t
D_0	100	$\mathrm{g-dry/m^2 s}$	initial mat density
D_L	176	$\mathrm{g-dry/m^2 s}$	limit mat density
E	Input data	h	photoperiod
E_{min}	2	h	minimal photoperiod
E_{op}	13	h	optimal photoperiod
g	Eq.	$1/\mathrm{d}$	growth rate for pH
g_{max}	0.26	$1/\mathrm{d}$	maximum intrinsic growth rate for pH
K_{IN}	604	$\mathrm{mg-N/L}$	N inhibition rate
K_{IP}	101	$\mathrm{mg-P/L}$	P inhibition rate
K_N	0.95	$\mathrm{mg-N/L}$	N saturation rate
K_P	0.31	$\mathrm{mg-P/L}$	P saturation rate
I_I	Input data	-	limitation factor for light saturation at 342 µmol m^{-2} s^{-1}
p	Input data	-	pH
R	0.62	$1/\mathrm{d}$	maximum intrinsic growth rate constant
r_i	Eq.	$1/\mathrm{d}$	intrinsic growth rate
$r_{i(E)}$	Eq.	$1/\mathrm{d}$	growth rate for photoperiod
$r_{i(P,N)}$	Eq.	$1/\mathrm{d}$	growth rate for phosphorus and nitrogen
$r_{i(pH)}$	Eq.	$1/\mathrm{d}$	growth rate for pH (corrected with model)
$r_{i(T)}$	Eq.	$1/\mathrm{d}$	growth rate for temperature
$r_{i,max}$	Eq.	$1/\mathrm{d}$	maximum intrinsic growth rate
r_s	Eq.	$1/\mathrm{d}$	specific growth rate
T	Input data	°C	temperature
t	Input data	s	retention time
T_{op}	26	°C	optimal temperature
θ_1	0.66	-	non-dimensional constant smaller than 1
θ_2	0.0025	-	non-dimensional constant smaller than 1
θ_3	0.0073	-	non-dimensional constant smaller than 1
θ_4	0.65	-	non-dimensional constant smaller than 1
α_E	0.42	$1/\mathrm{d}$	constant for the maximum intrinsic growth rate
$\alpha_{P,N}$	0.46	$1/\mathrm{d}$	constant for the maximum intrinsic growth rate
α_T	0.41	$1/\mathrm{d}$	constant for the maximum intrinsic growth rate

4. Photosynthesis model

The model used in the this thesis, is an adapted crop photosynthesis model from van Ooteghem (2007). This dynamic model used by van Ooteghem is a combined model from Farquhar et al. (1980) and Goudriaan et al. (1985) that both contain biochemical processes for leaf photosynthesis. Gaussian integration is used for the light interception in the different layers over the crop height. However, since the canopy of duckweed fronds will be assumed as one layer where no overlap occurs, this integration can be ignored.

4.1. Adjustments

The model first calculates the maximum gross assimilation rate ($P_{g,max}$) for one layer of leaves and then uses this equation to compute the maximum gross assimilation rate of every layer. Three layers are taken into account and are weighted differently, with a weight factor of 0.27, 0.44 and 0.28 for the top, middle and bottom layer, respectively. Then the assimilation rates are corrected for a sunlit and a shaded part and multiplied by a leaf area index (LAI). The LAI means the leaf area per unit ground surface. The optimal temperature in van Ooteghem (2007) is set to 25°C, which is for a tomato crop. For duckweed it is changed to 26°C, as observed by Ruigrok (2015) and Docauer (1983) as written in Landolt et al. (1987).
When cultivating duckweed in a pond, as it is done at the Ecoferm project, the value for LAI can be set to 1, since the canopy will be assumed as distributed evenly around the whole pond. Furthermore, the leaves will be positioned horizontally on the water surface and can observe as much direct as diffuse light (blockades like building structure elements are not taken into account). Also in this thesis, the canopy will be assumed as one layer where no overlap takes place. This means that the photosynthesis model does not have to be weighted over different layers, and the weight factor can be set to 1. Furthermore, only the sunlit part is important because only a very small part of the canopy will be shaded by building structures. This part will be ignored. It is assumed that there will be no shading from other leaves or building structure elements.

4.2. Adjusted model

The final carbon assimilation model for photosynthesis is computed from several equations for biochemical processes, which take part in carbon assimilation.

4.2.1. Net assimilation rate:

The net assimilation rate ($P_{n,max}$) is determined by the CO_2 limited rate for photosynthesis (P_{nc}) and the maximum endogenous photosynthetic capacity (P_{mm}).

$$P_{n,max} = \frac{(P_{mm} + P_{nc} - ((P_{mm} + P_{nc})^2 - 4\theta * P_{mm} * P_{nc})^{\frac{1}{2}})}{2\theta} \quad [mg/m^2s] \quad [29]$$

The degree of curvature will be determined by the parameter θ and is received from Goudriaan et al. (1985). The maximum endogenous photosynthetic capacity (P_{mm}) results from the molar mass of CO_2 and the maximum electron transport rate (J_{max}) that is computed by 2 intermediate variables (under influence of temperature (T_c and T_{26}) and some constants (R_g, S and H)), the maximum electron transport rate at the optimal temperature of 26°C , and the activation energy for J_{max}. The equations are described at the next page.

Maximum endogenous photosynthetic capacity:

$$P_{mm} = \frac{M_{CO2}}{4} * J_{max} \qquad [mg/m^2 s] \qquad [30]$$

Maximum electron transport rate:

$$J_{max} = J_{max26} * \frac{e^{E_J * X}}{D} \qquad [\mu mol/m^2 s] \qquad [31]$$

Intermediate variables:

$$X = \frac{T_c - T_{26}}{T_c * R_g * T_{26}} \qquad [mol/J] \qquad [32]$$

$$D = \frac{1 + \frac{e^{S*T_c - H}}{R_g * T_c}}{1 + \frac{e^{S*T_{26} - H}}{R_g * T_{26}}} \qquad [-] \qquad [33]$$

Besides the endogenous photosynthetic capacity, the net assimilation rate is also computed by the CO_2 limited rate for photosynthesis (P_{nc}), which needs the density for CO_2 (at time t) and the CO_2 concentration and the temperature (water) as input, and furthermore is calculated by the total resistance to CO_2 diffusion, which exists of the carboxylation, the stomatal and the boundary layer resistance to CO_2 diffusion. Furthermore, the CO_2 compensation concentration in absence of dark respiration rate is needed to determine P_{nc}. The values for the resistances to CO_2 diffusion and for the CO_2 compensation concentration are obtained from Fuhrer (1983) and Frost-Christensen and Floto (2007).

The equations are as follows:

CO_2 limited rate for photosynthesis:

$$P_{nc} = \frac{\rho_{CO_2 T_c}}{R_{tot\_CO2}} * (\max(CO_{2\alpha}, \Gamma) - \Gamma) \qquad [mg/m^2 s] \qquad [34]$$

The CO_2 density at a given temperature T_c can be determined as follows:

$$\rho_{CO_2}(T_c) = \rho_{CO_2}(T_0) * \frac{T_0}{T_c} \qquad [kg/m^3] \qquad [35]$$

Total resistance to CO2 diffusion:

$$R_{tot\_CO2} = R_{CO2} + R_{c\_CO2} \qquad [s/m] \qquad [36]$$

where R_{c_CO2} depends on the light intensity according to Fuhrer (1983) and R_{CO2} takes a constant value. Interpolating a table with given values for the carboxylation rate gives a table as follows:

Table 6: Carboxylation resistance in dependence of the light intensity (Fuhrer, 1983)

I_A (W/m^2)	$R_{c\,CO2}$ (s/m)
2500	1313
380	1313
150	1710
90	2461
60	4000
50	5299
0	11794

When not assuming the values given by the literature for the CO_2 diffusion resistances and CO_2 compensation resistance, the following computations take place (equation 37 to 43):

CO_2 compensation concentration:

$$\Gamma = \frac{K_C}{2K_O} * pO2_i * f_{OC} \qquad [\mu mol/mol\ (ppm)] \qquad [37]$$

where K_C and K_O are Michaelis-Menten constants for Rubisco carboxylation for CO_2 and O_2 respectively that are computed as follows:

$$K_C = K_{C,26} * e^{E_C * X} \qquad [s/m] \qquad [38]$$

$$K_O = K_{O,26} * e^{E_O * X} \qquad [\mu mol/mol] \qquad [39]$$

where E_C and E_O are activation energies for Rubisco carboxylation for CO_2 and O_2 respectively.
Total resistance to CO2 diffusion:

$$R_{tot\_CO2} = R_{CO2} + R_{c\_CO2} \qquad [s/m] \qquad [40]$$

where R_{CO2} is the stomatal resistance added to the boundary layer resistance and R_{c_CO2} is the carboxylation rate determined by another Michaelis-Menten equation (with influence from the O_2 partial pressure inside the stomata):

Effective Michaelis-Menten constant (CO_2):

$$K_M = K_C * \left(1 + \frac{pO2_i}{K_O}\right) \qquad [\mu bar] \qquad [41]$$

Maximum carboxylation rate:

$$V_{C,max} = V_{C,max,26} * e^{E_{VC} * X} \qquad [\mu mol/m^2 s] \qquad [42]$$

where E_{VC} is the activation energy of the maximum carboxylation rate and $V_{C,max,26}$ is the maximum carboxylation rate at optimal temperature T_{26}:

$$V_{C,max,26} = \rho_{Chl} * k_C * E_t \qquad [\mu mol/m^2 s] \qquad [43]$$

where the superficial chlorophyll density (ρ_{Chl}), the turnover number of RuP2 (carboxylase) (k_C) and the total concentration of enzyme sites (E_t)are multiplied with each other respectively.

4.2.2. Gross assimilation rate:

The gross assimilation for the whole canopy (P_g) is computed in a couple of steps. First it will be calculated for one leaf, then for the whole canopy under influence of the incoming sunlight corrected for the pond area. Again, shadows from building structure elements are not taken into account and the leaf area index (LAI) will be set on one, because the canopy will be assumed as homogeneous and horizontal on the water surface.

The gross assimilation for leaves can be calculated as follows:

$$P_{g,max} = P_{n,max} + r_{D\_uL} * M_{CO2} \qquad [mg/m^2 s] \qquad [44]$$

where the net assimilation rate and the respiration rate that is multiplied by the molar mass of CO_2 (which is taken as a constant for both day and night) are added to each other.

When we want to know what the gross assimilation will be for the whole canopy under influence of sunlight, first we determine the sunlight influence and then correct the model to the given area.
Gross assimilation under influence of sunlight:

$$P_{g,sun} = P_{g,max} * \left(1 - e^{-\frac{\varepsilon * I_A}{P_{g,max}}}\right) \qquad [mg/m^2 s] \qquad [45]$$

The sunlight correction takes exponent of the input light irradiance multiplied with the light use efficiency by photorespiration (ε) and divided by the maximum gross rate for leaves.

The light use efficiency can be calculated as follows:

$$\varepsilon = \psi * M_{CO2} * \frac{(\max(CO_{2\alpha}, \Gamma) - \Gamma)}{(4\ \max(CO_{2\alpha}, \Gamma) + 8\Gamma)} \qquad [\mu mol/J] \qquad [46]$$

where ψ is a conversion factor from J to e^-, corrected by the fraction of PAR (photosynthetically active radiation) absorbed by non-photosynthetic tissues multiplied by a conversion factor from J to photons:

$$\psi = \frac{1 - F_p}{2} * \xi \qquad [\mu mol/J] \qquad [47]$$

The final gross assimilation for the whole area is determined by a conversion factor (milligrams to kilograms) and the given area. The dark respiration rate will be subtracted from the gross assimilation rate because that is needed for the own metabolism of the duckweed plant and does not lead to the assimilation of CO_2.

$$Pg = \frac{R_{pH} * (P_{g,sun} - r_{D_{uL}} * M_{CO2})}{1 * 10^6 * A_{pond}} \qquad [mg/m^2 s] \qquad [48]$$

where R_{pH} is a correction for the pH. This is determined by an equation found by McLay (1976). In this thesis the equation determining the pH dependence for the S. polyrhiza species was used that gave an optimum at a pH of 7. The equation for L. minor should give an optimum by a value of 6.2. However, this was not the case (Appendix A), which lead to the use of the S. polyrhiza equation:

$$g = -1.0082 + 0.59297 * pH - 0.10831 * pH^2 + 0.00948 * pH^3 - 0.00034 * pH^4 \qquad [1/d] \qquad [49]$$

where the input data pH is used to calculate the growth per day as a function of the pH level, the correction factor (R_{pH}) used in the model can be calculated as follows:

$$R_{pH} = \frac{g}{g_{max}} \qquad [1/d] \qquad [50]$$

g_{max} is determined by McLay (1976) which describes the peak rate of increase.

The already known harvest data is given in kilograms per day. This means that we have to multiply the gross assimilation rate by a conversion factor, which results in:

$$P_{g,day} = Pg * 3600 * 24 \qquad [mg/m^2 s] \qquad [51]$$

At the next pages, Table 7 gives an overview of the parameters that are used to determine the photosynthesis model is given.

Table 7: Overview of the parameters used to determine the photosynthesis model

Name	Value	Unit	Content
A_{pond}	880	m^2	area of the cultivation pond (van den Top, 2014)
$CO_{2\alpha}$	Input data	$\mu mol/mol\ (ppm)$	CO_2 concentration indoor air below screen
D	Eq.	-	intermediate variable
E_C	59356	J/mol	activation energy K_C Rubisco carboxylation*
E_D	66405	J/mol	activation energy r_D dark respiration rate
E_J	37000	J/mol	activation energy J_{max} maximum electron transport rate
E_O	35948	J/mol	activation energy K_O Rubisco oxygenation*
E_t	87	$\mu mol/g$	total concentration of enzyme sites*
E_{VC}	58520	J/mol	activation energy VC max maximum carboxylation rate*
f_{OC}	0.21	-	ratio VO_{max}/VC_{max}
F_p	0.1	-	fraction PAR absorbed by non-photosynthetic tissues
g	Eq.	$1/d$	growth per day as a function of the pH level
g_{max}	0.27	$1/d$	maximum growth per day as a function of the pH level (McLay, 1976)
H	220000	J/mol	constant for temperature dependency J_{max}
I_A	Input data	W/m^2	absorbed radiation
J_{max}	Eq.	$\mu mol/m^2s$	maximum electron transport rate
J_{max26}	467	$\mu mol/m^2s$	maximum electron transport rate at 26°C
K_C	Eq.	s/m	Michaelis-Menten constant Rubisco carboxylation (CO_2)
k_C	2.5	$1/s$	turnover number of RuP2 (carboxylase)*
K_M	Eq.	$mbar$	effective Michaelis-Menten constant (CO_2)*
K_O	Eq.	$\mu mol/mol$	Michaelis-Menten constant Rubisco oxygenation (O_2)*
M_{CO2}	$44*10^{-3}$	kg/mol	molar mass CO_2
P_g	Eq.	kg/s	maximum gross assimilation whole area per second
$P_{g,day}$	Eq.	kg/d	maximum gross assimilation whole area per day
$P_{g,max}$	Eq.	mg/m^2s	maximum gross assimilation rate leaves
$P_{g,sun}$	Eq.	mg/m^2s	gross assimilation rate sunlight influence
P_{mm}	Eq.	mg/m^2s	maximum net assimilation rate
$P_{n,max}$	Eq.	mg/m^2s	maximum endogenous photosynthetic capacity
P_{nc}	Eq.	mg/m^2s	CO_2 limited rate of net photosynthesis
pH	Input data	-	pH level of the pond

$pO2_i$	210	Mbar	O_2 partial pressure inside stomata*
R_{b_CO2}	160	s/m	leaf boundary layer resistance to diffusion of CO_2*
R_{c_CO2}	Eq.	s/m	carboxylation resistance
r_{D_uL}	$60/M_{CO2}$	$\mu mol/m^2 s$	leaf dark respiration rate (Fuhrer, 1983)
r_{D26_uL}	60	$\mu mol/m^2 s$	dark respiration rate at 26°C
R_{pH}	Eq.	$1/d$	correction factor for pH level
R_s	62.5	s/m	stomatal resistance (constant) (Fuhrer, 1983)
R_{cut}	$239*10^3$	s/m	cuticular resistance (Frost-Christensen and Floto, 2007)
R_{s_CO2}	Eq.	s/m	leaf stomatal resistance to diffusion of CO_2*
R_{tot_CO2}	Eq.	s/m	total resistance to CO_2 diffusion
R_{CO2}	Eq.	s/m	stomatal resistance + boundary layer resistance to CO_2 diffusion
R_g	8.314	$J/mol * K$	gas constant
S	710	$J/mol * K$	constant for temperature dependency J_{max}
T_{26}	273.15+26	K	temperature of 26°C
T_C	273.15+Input data (°C)	K	(state) temperature crop
$V_{C,max}$	Eq.	$\mu mol/m^2 s$	maximum carboxylation rate*
$V_{C,max,26}$	Eq.	$\mu mol/m^2 s$	maximum carboxylation rate at 26°C*
X	Eq.	mol/J	intermediate variable
Γ	36.5	$\mu mol/mol\ (ppm)$	CO_2 compensation concentration in absence of dark respiration (Fuhrer, 1983)
ε	Eq.	mol/J	light use efficiency by photorespiration
θ	0.71	-	degree of curvature $P_{n,max}$
ξ	4.59	$\mu mol/J$	conversion factor from J to photons
ρChl	0.45	g/m^2	superficial chlorophyll density*
$\rho CO_2 T_0$	1.98	kg/m^3	density CO_2 at 0°C
$\rho CO_2 T_{26}$	Eq.	kg/m^3	CO_2 density at 26°C
$\rho CO_2 T_c$	Eq.	kg/m^3	CO_2 density at T_c (gas law)
ψ	Eq.	$\mu mol/J$	conversion factor, J to e^-

*only when the parameters from literature are ignored

4.3. Sensitivity analysis

A sensitivity analysis is used to give insight in which parameters should be used for parameter estimation to give a better fit to the available growth data. By using a Fisher information matrix, the sensitivity of the corresponding parameters will be found what gives insight in what parameters are most depending for the photosynthesis model. Instead of changing the parameter values, a multiplication factor (parameters θ) with a nominal value of 1 is used to prevent scaling problems.

The local sensitivities for the parameters θ are given by the following equation:

$$\dot{x_\theta} = \frac{\partial f(t,u,\theta)}{\partial \theta} \quad [52]$$

With time t and inputs u, f describes the photosynthesis model.

The function above is integrated from the initial time to the final time for 1 month with time steps of 10 minutes. The Runga-Kutta fourth order integration algorithm is used with nominal parameter values $\bar{\theta}$:

$$x(t,x,\bar{\theta}) = \int_{t_0}^{t_f} f(t,u,\bar{\theta})\, dt \quad [53]$$

A variation of 10% on the parameters was used. Then, an Euler forward integration method is used to determine the trajectories of the sensitivities:

$$x(t,x,\bar{\theta}) = \int_{t_0}^{t_f} \left\{\frac{\partial f(t,u,\theta)}{\partial \theta}\right\} dt \quad [54]$$

With the difference in output (∂y) a Jacobian matrix was obtained to take the derivative of Y to each of the parameters:

$$\partial y = y - y_{nom} \quad [55]$$

$$J = \begin{pmatrix} \frac{\partial y(1|\boldsymbol{\theta})}{\partial \theta_1} & \frac{\partial y(1|\boldsymbol{\theta})}{\partial \theta_2} & \cdots & \frac{\partial y(1|\boldsymbol{\theta})}{\partial \theta_{n_p}} \\ \frac{\partial y(2|\boldsymbol{\theta})}{\partial \theta_1} & \frac{\partial y(2|\boldsymbol{\theta})}{\partial \theta_2} & \cdots & \frac{\partial y(2|\boldsymbol{\theta})}{\partial \theta_{n_p}} \\ \vdots & \vdots & & \\ \frac{\partial y(N|\boldsymbol{\theta})}{\partial \theta_1} & \frac{\partial y(N|\boldsymbol{\theta})}{\partial \theta_2} & \cdots & \frac{\partial y(N|\boldsymbol{\theta})}{\partial \theta_{n_p}} \end{pmatrix} \quad [56]$$

Eventually, the Fisher information matrix will be used to determine for what parameter the model is the most sensitive. The Fisher information matrix will be computed as follows:

$$F = \int_{t_0}^{t_f} x_\theta(t)^T * Q_F^{-1} * x_\theta(t)\, dt \quad [57]$$

where Q_F is a weighing matrix with values of $\frac{1}{\sigma^2}$ on the diagonal of the matrix and x_θ the cumulative sum of the computed Jacobian matrix. The outcomes of the Fisher information matrix display the sensitivities of the parameters θ. The higher the corresponding value, the more sensitive the parameter θ will be for the model.

4.4. Parameter estimation

When the sensitivities of the parameters are determined, parameter estimation can be performed. The pathway that is used to determine the estimated parameters is presented in Figure 11.

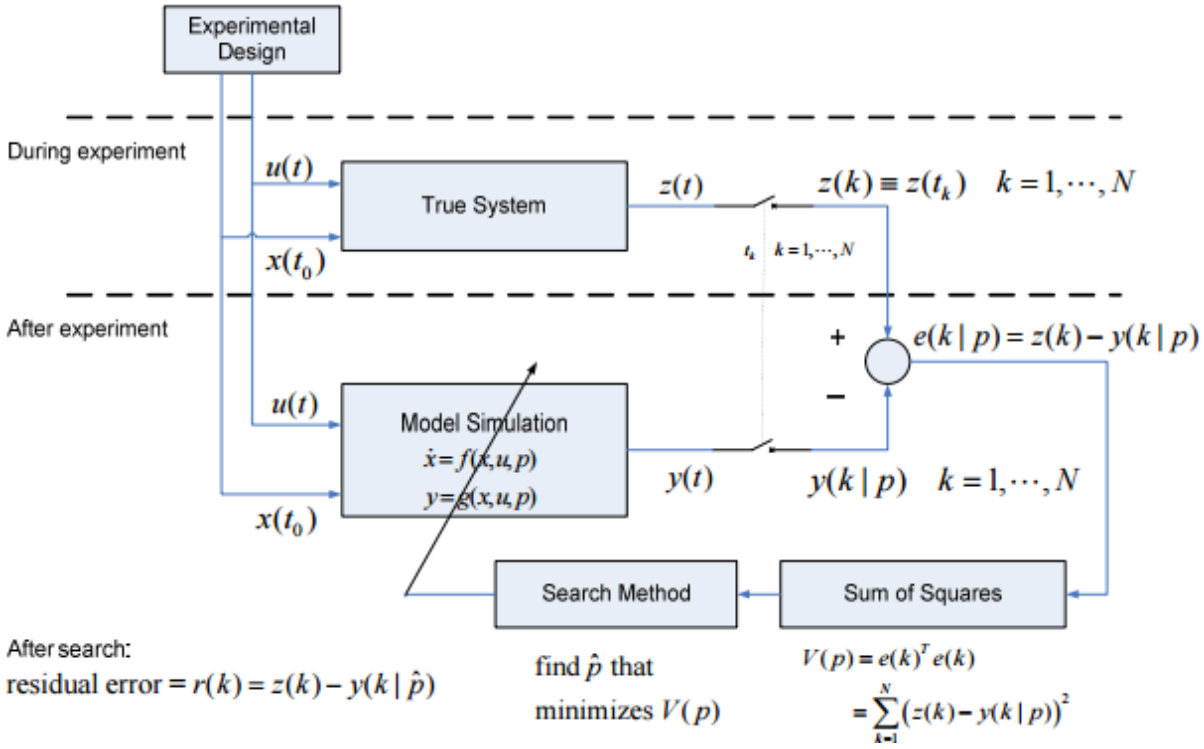

Figure 11: Parameter search method (van Ooteghem and van Willigenburg, 2014-2015)

Here $z(k)$ represents the data outcomes and $y(k|\mathbf{p})$ the model outcomes with the parameters **p**. The error $\big(e(k|\mathbf{p})\big)$ that is used to calculate the sum of squared errors can be found by:

$$e(k|\mathbf{p}) = z(k) - y(k|\mathbf{p}) \qquad k = 1, \dots, N \qquad [58]$$

After the error vector is found, the sum of squared errors $\big(V(\mathbf{p})\big)$ that has to minimized can be found using the following equation:

$$V(\mathbf{p}) = \sum_{k=1}^{N} \big(z(k) - y(k|\mathbf{p})\big)^2 \qquad k = 1, \dots, N \qquad [59]$$

or

$$V(\mathbf{p}) = e(\mathbf{p})^T e(\mathbf{p}) \qquad [60]$$

With a function that tries to adapt the parameters in a way that a minimal error is found *(fminsearch)* in MATLAB, the error $\big(e(k|\mathbf{p})\big)$ can be found that estimates the parameters that fit the model best, this step can also be written as the argument of function V that minimizes **p**:

$$\hat{\mathbf{p}} = arg\min\big(V(\mathbf{p})\big) \qquad [61]$$

The parameters $(\hat{\mathbf{p}})$ that are found from the minimization function, can be implemented in the model what should give a better representation of the given harvest data $\big(z(k)\big)$ and a smaller error $\big(e(k|\mathbf{p})\big)$.

5. Results

The previous photosynthesis model is used to predict the growth of the Ecoferm duckweed with the inputs derived from datasheets of the months May, September and October (2015).

5.1. Fisher information matrix

The Fisher information matrix in Table 8 gives insight in the sensitivities of the parameters for the model. The matrix below is calculated for the photosynthesis model with the parameters J_{max26}, E_J, R_s, R_{cut}, θ, Γ, T_{26}, r_{DuL} and R_{cCO2}.

Table 8: Fisher information matrix of the photosynthesis model with the used parameters. The bold numbers show the sensitivities of each parameter and the non-bold numbers show mutual relations. Green and Red colours indicate positive and negative relations respectively

$\tilde{F} = 10^{14}$

2.508	0.042	1.010	0.740	-0.435	1.070	1.017	-0.762	0.132	0.575	J_{max26}
	0.037	0.043	0.002	0.009	0.001	-0.010	-0.017	0.002	0.002	E_J
		0.433	0.290	-0.153	0.422	0.387	-0.306	0.053	0.229	R_s
			0.222	-0.133	0.321	0.308	-0.223	0.039	0.173	R_{cut}
				0.101	-0.187	-0.193	0.140	-0.021	-0.096	θ
					0.467	0.445	-0.320	0.057	0.252	Γ
						0.434	-0.307	0.053	0.237	F_p
							0.241	-0.038	-0.167	T_{26}
								0.009	0.033	r_{DuL}
									0.139	R_{cCO2}
J_{max26}	E_J	R_s	R_{cut}	θ	Γ	F_p	T_{26}	r_{DuL}	R_{cCO2}	

From this matrix, we can choose what parameters are going to be used for parameter estimation. We can see that J_{max26} is the most determining parameter for the model.
Other parameters that are qualified for parameter estimation are R_s, R_{cut}, Γ and θ. F_p is ignored for the parameter estimation because adaptions in this parameter did not lead to desirable results. θ is described as a curvature factor in the model and adjustments to this parameter lead to great variations in the model. Nevertheless, although the sensitivity analysis did not show a big sensitivity on this parameter, θ is taken into account with the parameter estimation.

After the parameter estimation is performed, the nominal values for the parameter values (which were set to 1) are as described in the table onder:

Table 9: Parameter values after parameter estimation

pars (#)	parameter	nominal value	estimated value
1	J_{max26}	1	1.00812
2	R_s	1	0.99266
3	Γ	1	1.01476
4	θ	1	1.4339
5	R_{cut}	1	0.98945

The parameters that were selected for the parameter estimation are correlated with each other. The strength of this correlation is showed in Table 10.

Table 10: Correlations between the selected parameters for parameter estimation. The colours indicate the sensitivity of each parameter. Green means very sensitive, yellow means less sensitive

	J_{max26}	R_s	Γ	θ	R_{cut}
J_{max26}		++	++	-	+
R_s	++		+	-	+
Γ	++	+		-	+
θ	-	-	-		-
R_{cut}	+	+	+	-	

From this table we can see that the parameter J_{max26} has a relative strong positive correlation with R_s, and Γ (also for F_p but this parameter was not included in the set parameters for parameter estimation). It also does have a reasonable correlation with parameter R_{cut} and it has a negative correlation with the parameters θ. This parameter (θ) has a negative correlation with every other parameter selected. Between the parameters R_s, Γ and R_{cut}, a positive correlation was found.

5.2. Input data

The model uses four inputs to predict the growth. Data of the light intensity, temperature and CO_2 concentration every minute of the day for a whole month is available for the months May, September and October. The pH level was measured just a few times each month. To reduce computing time, data for every 10 minutes is used. Also because Lemna plants need 3 to 6 minutes of illumination to induce activity in photosystem-II (Landolt et al., 1987), 10 min data suits the dynamics of the plant.

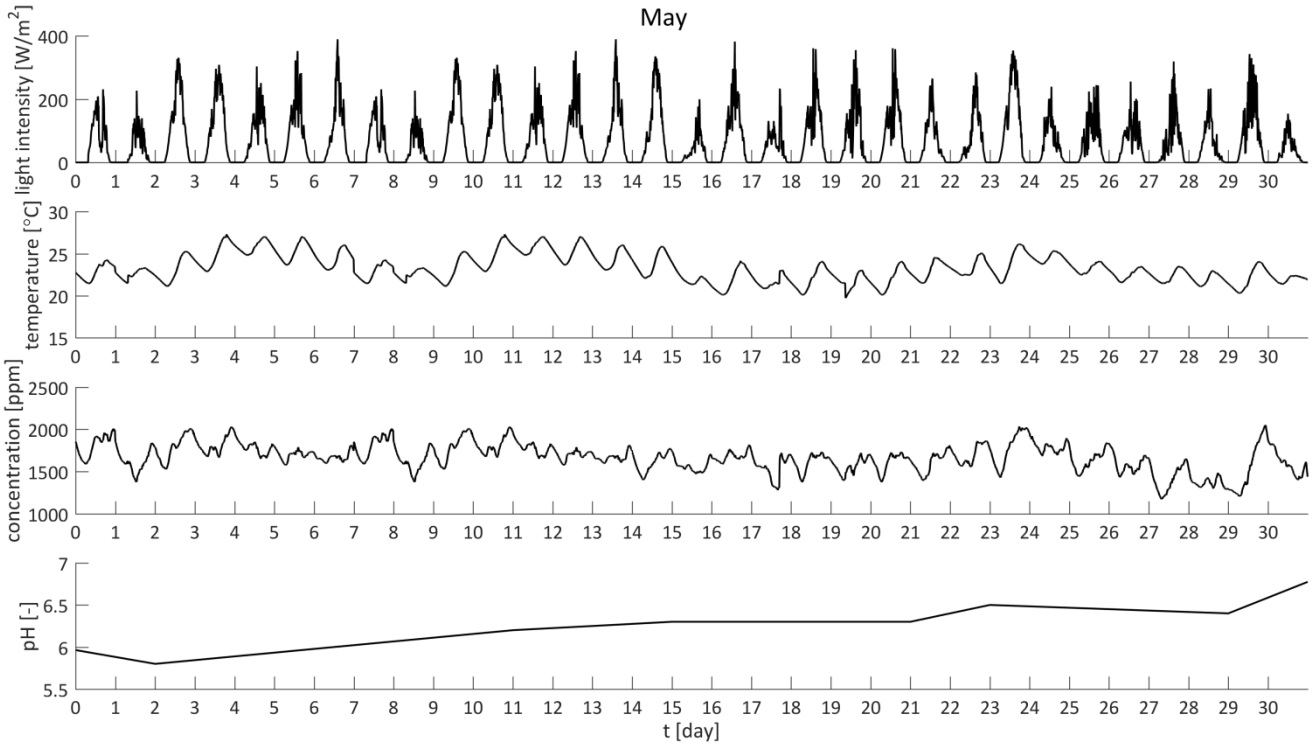

Figure 12: Input data for the month May with light intensity, temperature, CO2 concentration and pH from top to bottom

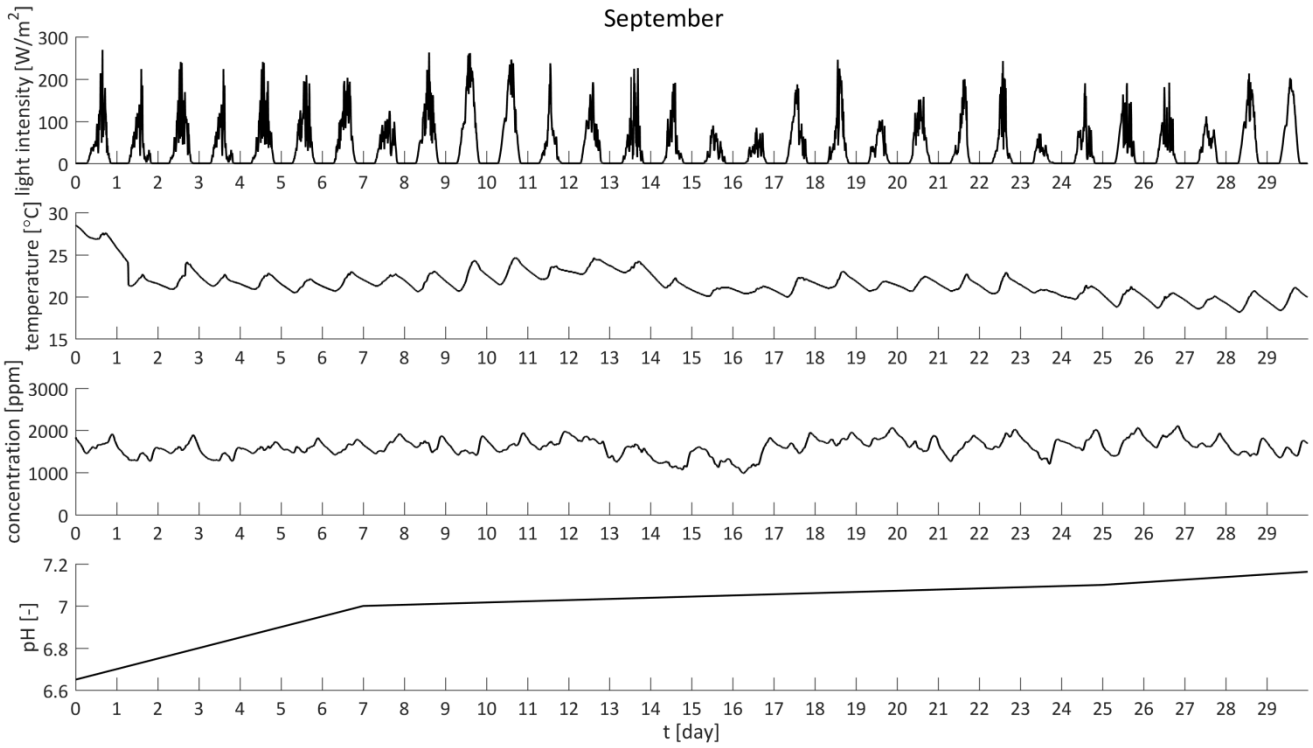

Figure 14: Input data for the month September with light intensity, temperature, CO2 concentration and pH from top to bottom

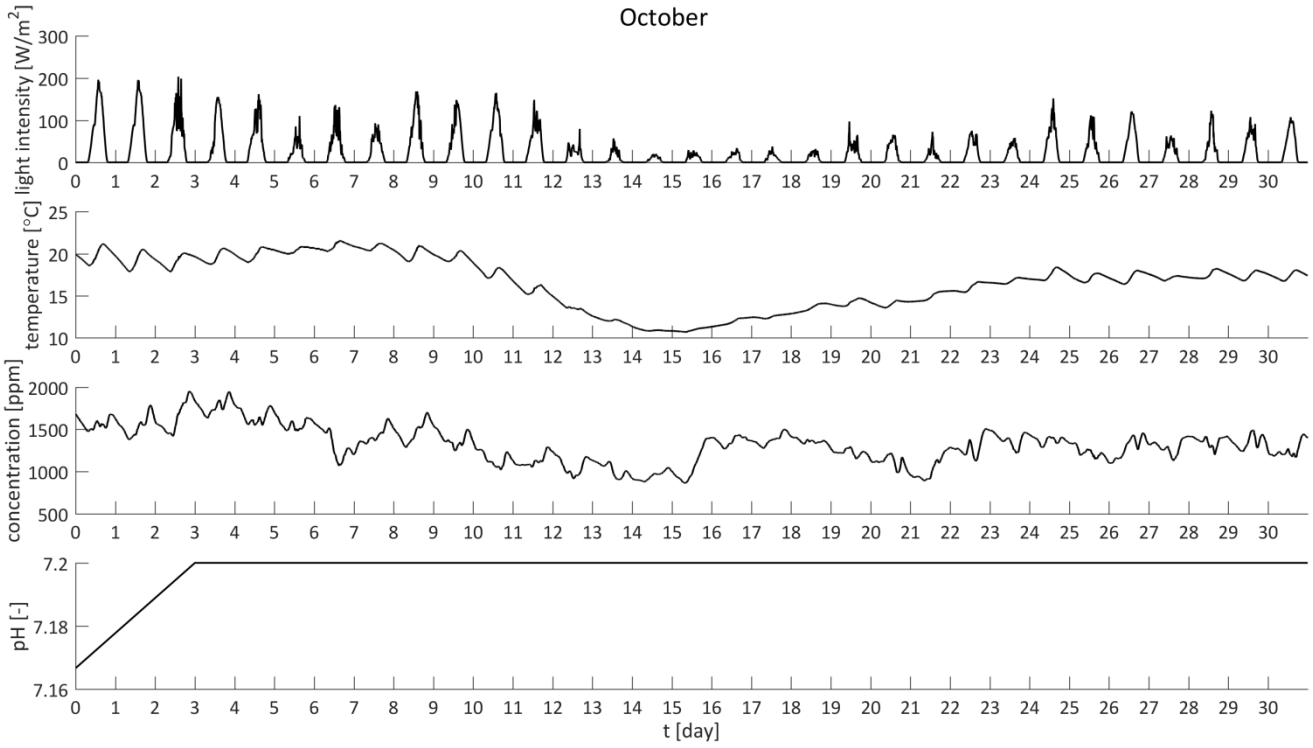

Figure 13: Input data for the month October with light intensity, temperature, CO2 concentration and pH from top to bottom

5.3. Model Results

First, the model is performed with parameters found in literature and assumed from the original model used by (van Ooteghem, 2007). Then, parameter estimation was used to give insights in how the parameters had to be chosen to give the best possible setting that lead to a best fit in general (for the months May, September and October).

Figure 15 below, and Figure 16 and Figure 17 at the next page show the cumulative growth of both the model (line and points at harvest time) and the harvest data before and after the performed parameter estimation. Harvest data is only available for the harvesting with corresponding days, an assumption is made that every harvest is performed at 3 o'clock in the afternoon.

Model results and input data for the other months that were not suitable for the validation of this model are shown in Appendix B.

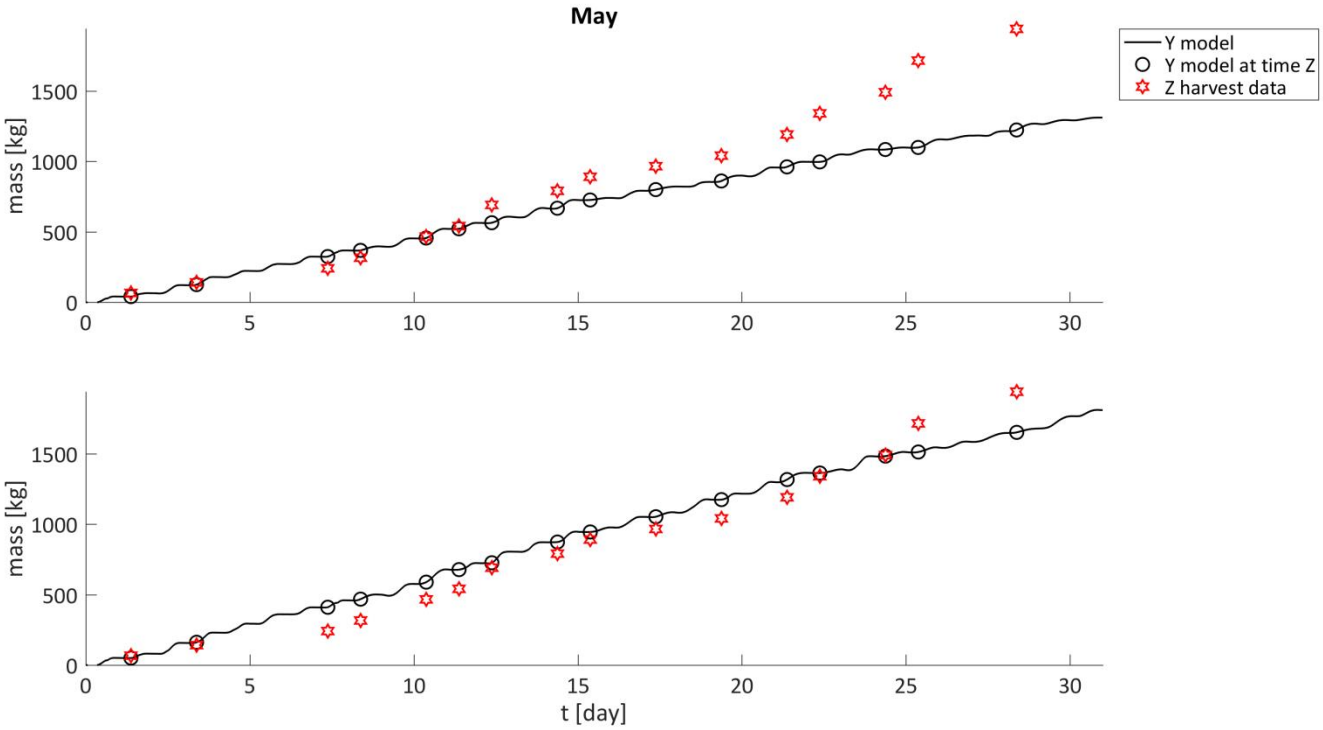

Figure 15: Cumulative production of duckweed at Ecoferm for the month May. The figures describe the model in comparison with the harvest data without and with parameter estimation (above and below respectively)

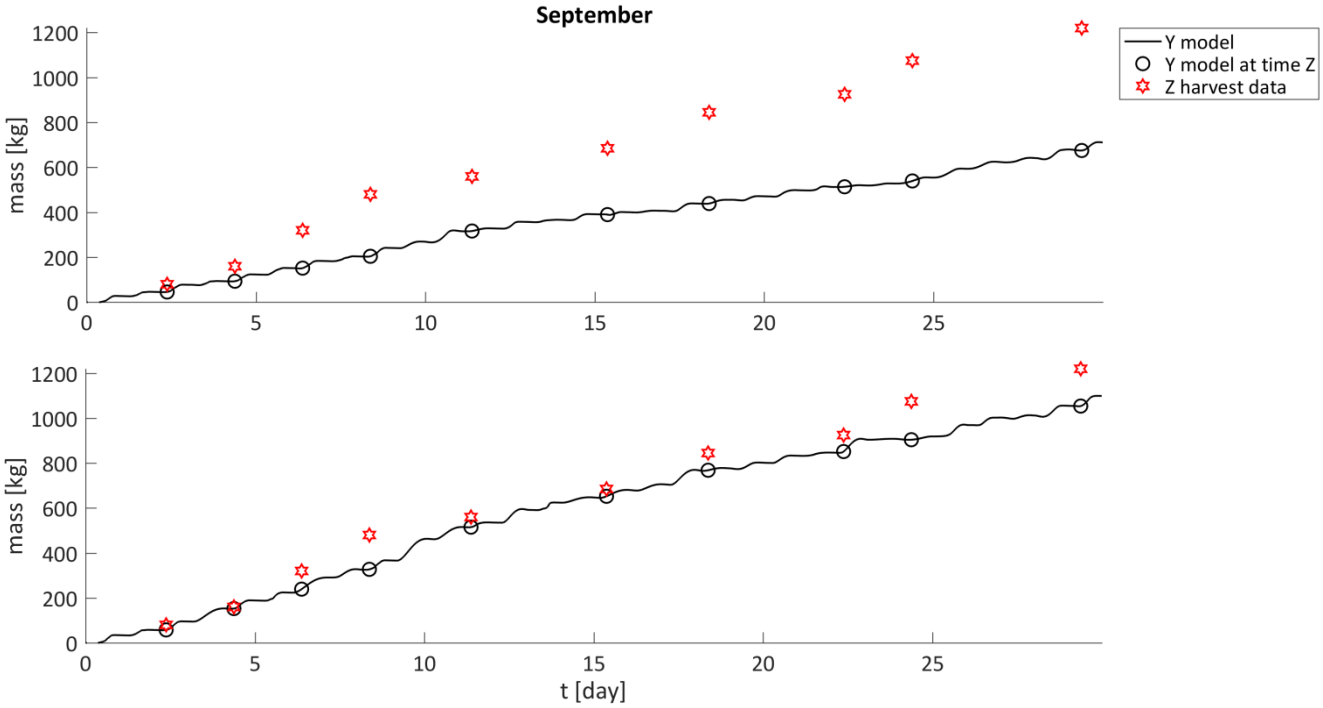

Figure 16: Cumulative production of duckweed at Ecoferm for the month September. The figures describe the model in comparison with the harvest data without and with parameter estimation (above and below respectively)

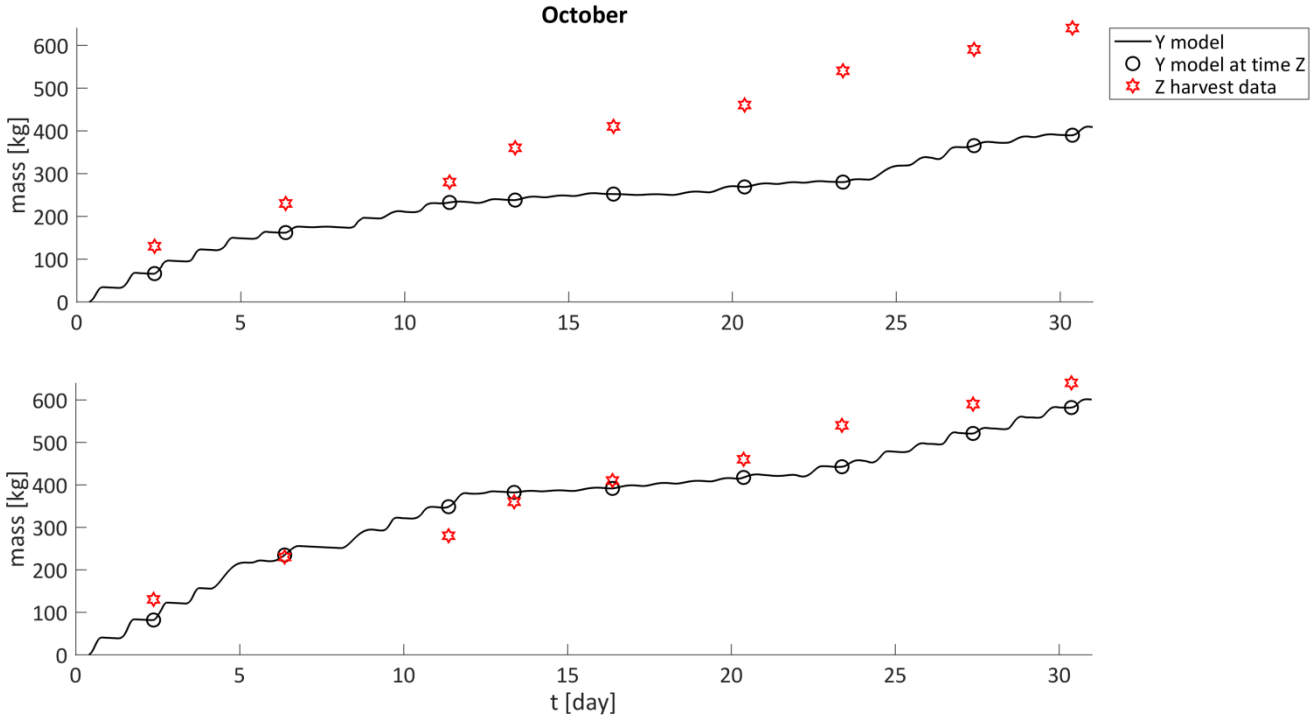

Figure 17: Cumulative production of duckweed at Ecoferm for the month October. The figures describe the model in comparison with the harvest data without and with parameter estimation (above and below respectively)

With corresponding sum of squared errors $(V(\mathbf{p}))$ before and after the parameter estimation:

Table 11: Sum of squared errors before and after the parameter estimation was performed for the months May, September and October. Also the change between the SSE before and after is given

Month	Sum of squared errors $(V(\mathbf{p}))$		
	Before	After	Change
May	1.36E+06	2.63E+05	-81%
September	1.17E+06	1.01E+05	-91%
October	2.68E+05	2.74E+04	-90%

The daily production is given in Figure 18 below, and Figure 19 and 20 at the next page. For comparison, the harvest data points per time step are added (the harvest moments were not evenly distributed over a fixed amount of time). For a more detailed overview about the sum of squared errors, Appendix C can be considered.

The simulated daily growth of duckweed contains data that reaches below zero. In chapter 2.2.7 respiration is explained as the sum of all metabolisms. This means that at night, the growth can be below zero.

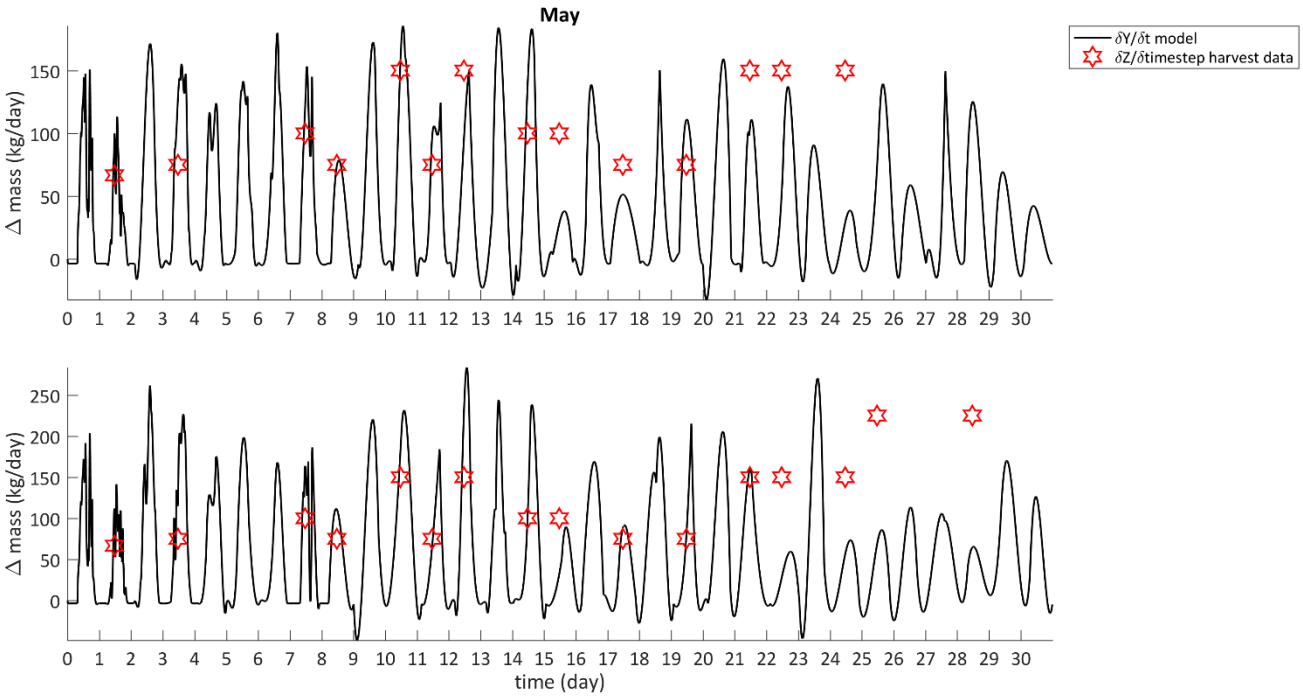

Figure 18: Daily production of duckweed at Ecoferm for the month May. The figures describe the model in comparison with the harvest data without and with parameter estimation (above and below respectively)

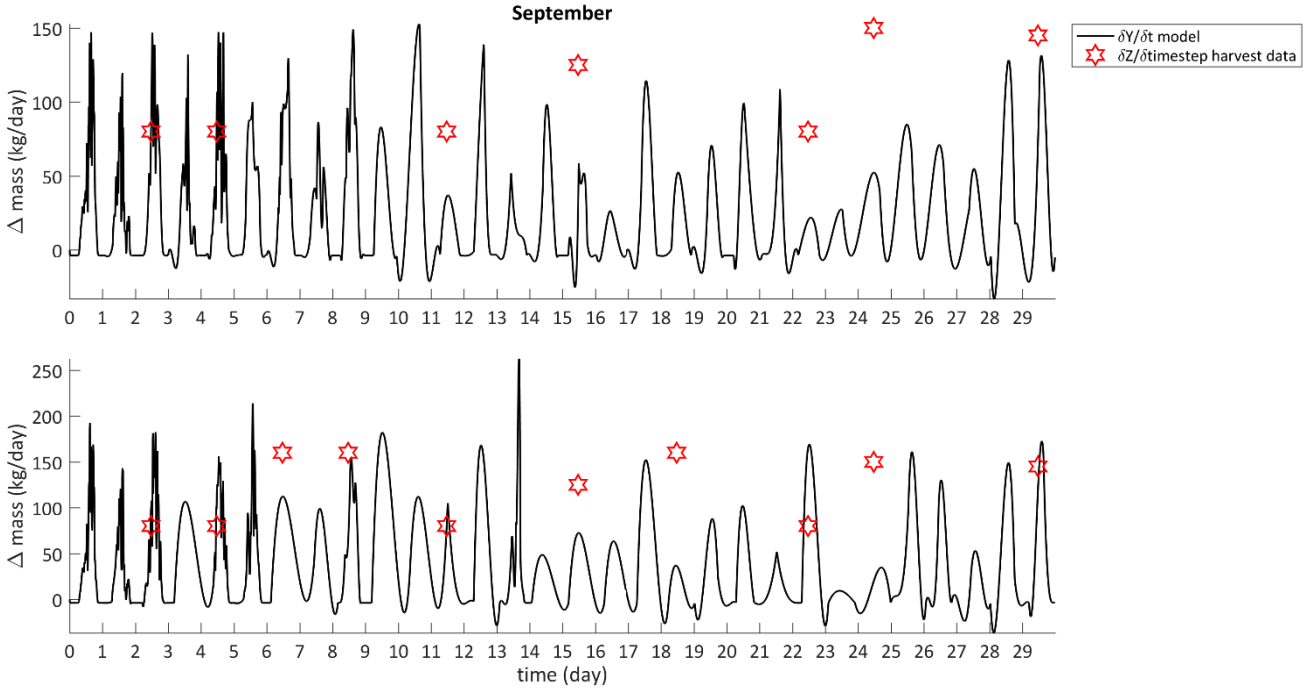

Figure 19: Daily production of duckweed at Ecoferm for the month September. The figures describe the model in comparison with the harvest data without and with parameter estimation (above and below respectively)

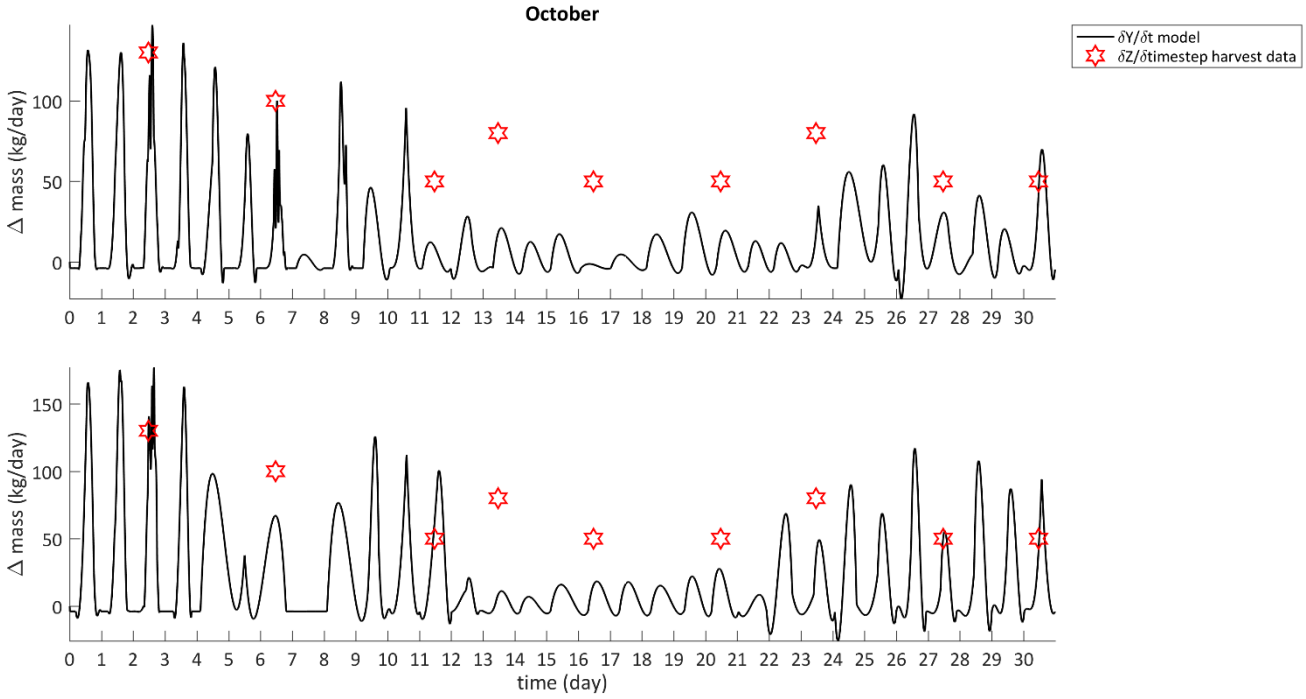

Figure 20: Daily production of duckweed at Ecoferm for the month October. The figures describe the model in comparison with the harvest data without and with parameter estimation (above and below respectively)

Table 12: Total production of each month (kg). Note: the last day of the harvest data corresponding the month May is not equal to the last day of the month. The model data is compared with the last day of the harvest data.

Month	Original (kg)	Fit (kg)	Harvest data (kg)	Deviation original	Deviation fit	Improvement
May	1223.951	1653.053	1941.667	37%	15%	22%
September	675.141	1054.113	1220.000	45%	14%	31%
October	389.433	581.904	640.000	39%	9%	30%

At the table boven the total production during one month is shown. Also deviations between the model before and after the parameter estimation with the harvest data are shown. The total production of the harvest data is the sum of all the data points. This means that for the month May, the total production cannot be shown because only data until the 29th (of 31) day is available.

6. Discussion

6.1. Cultivation data

The data used in this report is gained in the year 2015. The cultivation started in April and ended in the month October. From November until March, environmental factors make it impossible to cultivate duckweed in the way it is done now (no extra heating or artificial lighting). However, for the month April, no harvest data was available. For the remaining months where cultivation took place, both harvest and input data were available. However, for the months July (325 of the 4464 data points) and August (1554 of the 4464 data points) data for CO_2 concentration was limited to 2100ppm where possibly more was available in the greenhouse however due to (assuming) a limiting measuring device it was not possible to measure the actual amount of CO_2 available in the greenhouse. In the second week of the month June, a huge loss of duckweed occurred due to high temperatures and a low oxygen level. Also in this month the problem with the CO_2 concentration mentioned above occurred (800 of the 4320 data points). This means that these months are not useful for the model validation. The months May, September and October were suitable for the validation of the validation of the model, which is unfortunate because of the seven cultivation months, only three are left for validation.

The amount of measurements for the pH level was quite limited, which lead to a not so clear representation of the pH level in the pond for every 10 minutes.
For the temperature input data only the water temperature is used because it is assumed that this temperature is most determining for the duckweed temperature. This temperature fluctuates less the air temperature. However, while duckweed is a floating plant, the air temperature could also influence the duckweed temperature. This is not takin into account in this report because it is not known in what ratio the water and air temperature determine the duckweed temperature.
Nutrients are not taken into account as input data in this report because it is assumed that they are held constant by adding manure and trace elements to the pond. However, it maybe can improve the model when nutrient data is implemented.

Furthermore, the growth area is held constant. This may not always be the case because the duckweed fronds are pumped through the pond to maintain a homogeneous distribution. While the pond is not a circle, it can be possible that in the corners the duckweed will not be distributed evenly which can influence the growth.

6.2. Species

During the cultivation period, the family Kroes wrestled with some factors that were fatal for the duckweed growth and induced the death of duckweed described in paragraph Ecoferm situation. The main reason for the duckweed loss was the high temperature in the greenhouse. To prevent this and to maintain the growth of duckweed, adiabatic cooling was realised and plastic shading sheets were installed. After these adaptions, the duckweed could grow again. Adiabatic cooling was chosen above the plastic shields because the direct incoming sunlight was blocked when the shields were installed what reduces the duckweed growth.

One major adaption that also occurred to maintain the growth was the change of the dominant species that grows in the pond. Although some growth rates (like phosphorus and nitrogen source) do not differ much between the L. minor and the S. polyrhiza species, other growth rates like temperature or light intensity do differ. This makes the validation of the model difficult because during the

cultivation (second week of the month June) period the dominant species (with different optima for growth factors) changed from L. minor to S. polyrhiza after extra aeration in the pond was performed.

6.3. Diffuse lighting

According to chapter 2.3 it does not matter how much of the incoming light is scattered in accordance to the photosynthetic capacity of the duckweed canopy. It is assumed that the leaf area index is 1, and equally distributed over the whole pond. Also is assumed that the canopy is not separated into different vertical layers that can induce shading. The incoming light at one meter above the canopy is used as input data not considering a direct or diffuse component.

6.4. Parameter estimation

The month October showed the most fluctuations in input data; however the month May contained the most harvest data points, which made it most suitable for comparison. Nevertheless, the input data for the month May was very monotone and contained little variation. This lead to a position where it was possible to fit the model for the month May in a good way, but results for the months September and October were too bad for assumption. For the months October and September, it was also possible to give a good fit for each separate month. When the parameter set that was found was used on other months, it did not give desirable results. Eventually, taking the month September as starting point in combination with small adjustments to the chosen parameters gave desirable results that suited each selected month.

It was possible for each month to give a better fit to the available data. Again, using the values for the parameters found for one month gave bad results for the other one. The case of this thesis was to find a good prediction for any given input data what means that it cannot be month dependent but has to be able to predict any type of input.

6.5. Photosynthesis model

The adjusted photosynthesis model is able to predict the total production of duckweed at the Ecoferm to 9% (October) in comparison with the available harvest data. It is not clear how accurate the available harvest data is. Also the harvest time is not known, what leaded to an assumption that on a harvest day, the duckweed would be harvested at 3 o'clock in the afternoon. The time between two harvest times is not fixed. Apparently, when a maximum mat density (4.2.2) was reached, harvest would take place. It is again not clear if the maximum and minimum mat density are realised.

The sums of squared errors of the 3 months are greatly reduced after the parameter estimation was performed. Still, they amount a big value. The parameter estimation is performed with the sum of squared errors of the difference between the cumulative values of the harvest data and the corresponding model results. While the production per time step can contain big amounts, the sum of squared errors can become large. Especially when the cumulative production is used instead of the error of the daily production. The sum of squared errors for the cumulative production was chosen instead of the daily error because that resulted in a better fit. Errors of the daily production and the cumulative production of the models before and after the parameter estimation are shown in Appendix C.

Looking at the cumulative growth for each month, we can see that the month May resulted in a more linear growth. This can be explained by the fact that the month May contained a rather homogeneous input data with small variations. If we look at for example the month October, we see a temperature

and light intensity drop from day 11 until day 24. In the model, this is reproduced as little to no growth. Furthermore, we can see that particularly the temperature and light intensity have big influence on the model growth. From the input we can see that when the light intensity rises or drops, the temperature shows the same effect. CO_2 concentration has less influence which can be explained by the amount of CO_2 available in the greenhouse. There is always at least 1000 ppm of CO_2 available in the greenhouse, which is more than normal environmental situations (350-400 ppm). This means that there is always more than enough CO_2 available for carboxylation. It is not known how much the big amounts (above 2100 ppm) influence the model, therefore the months July and August were left out.

Predictions with deviations from 9% to 14% to the given harvest data were found with this set of parameters. Only three months were suitable for the model validation. The duckweed at Ecoferm was cultivated for seven months in 2015, this means that less than halve of the cultivation period can be used for model validation. Less data makes it harder to predict because it is possible that some environmental influences are not approximated correctly.

Conclusion

In 2015, the Ecoferm project was still in a development stage. The input data was not usable for every month what made it harder to give a good prediction of the duckweed production at the greenhouse. Still, three months had both good input and harvest data suitable for validation. This leaded to a prediction to at most 9% deviation of the real data what answers the research question of this report:

How does a photosynthesis model match the production of duckweed produced on Ecoferm?

What is known now, is that the model can predict the production of duckweed from 86% up to 91% of the corresponding harvest data. This is still not a correct reproduction of the real harvest data but it gives a good indication. Possible when more input and harvest data distributed over more periods of time are given, this can lead to a better prediction of the duckweed growth no matter what time of the year.

The answers on the three subquestions are as follows:

1. *How can we implement the available inputs into the model?*

The model takes four inputs to predict the production of duckweed: *light intensity, temperature, CO_2 concentration* and *pH level*. The model contains several processes that describe the photosynthesis. To perform photosynthesis, an amount *light intensity* is needed for the induction of Photosystem-II. Also, CO_2 carboxylation will take place and oxygen and sugar will be formed. The sugar will be stored or used for growth. Duckweed species can survive from *temperatures* from 5°C to 40°C depending on the species. The temperature is implemented in the model using an intermediate variable that describes the temperature at time *t* and the optimal temperature. One can say that the L. minor species survives better in cold climates and the S. polyrhiza species survives better in the winter. The same holds for pH levels. At the Ecoferm greenhouse *pH levels* have to be managed between the minimum and maximum levels to maintain duckweed growth. The pH levels are implemented in the model using a correction factor that uses the growth rate corresponding pH levels and the maximum growth rate.

2. *How can we validate the model?*

The model is implemented to simulate a period of one month. Harvest and input data was also available for each month. To validate the model, the cumulative real harvest data was compared with the cumulative production data of the model at the corresponding time harvest time. It was clear that some parameter estimation had to be used while the original model with assumed parameters did not give a clear prediction. With the right parameter values, errors between harvest and model data could be reduced with leaded to a better indication of the duckweed yield.

3. *Does the model represent the actual production?*

The model represents the actual production to at least 86%. It is possible to give a better prediction for every month separately, but that is not the case of this report. The case of this report is to give a good prediction of the growth of duckweed with the given parameters described above.

7. Recommendations

Environmental influences of three months were useful for the validation of the photosynthesis model. While the duckweed was cultivated from April until October only good data was available for the months May, September and October. It would be useful to compare the model with more data over a larger period of time to give better insight in how the parameter values have to be chosen for a better production prediction. Also, nutrients are now kept constant but this may not always be the case. The calves manure does not contain always contain the same amount of nutrients so it can be useful to implement the addition of nutrients to the pond to the model. Furthermore, the growth area is kept constant to 180 m^2 with an even distribution over the whole pond. However, it could be that some areas are less favourable for the growth. Finally, more insights about harvesting like mat density (beginning and ending of each harvesting period) and at what time of the day the duckweed was harvested could help giving a better prediction of the duckweed growth.

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1. Appendices

Appendix A

Correction functions for the pH levels, no maximum at 6.2 for the equation gained from (McLay, 1976) for Lemna minor. The equation for Spirodela polyrhiza does show a maximum at 7.

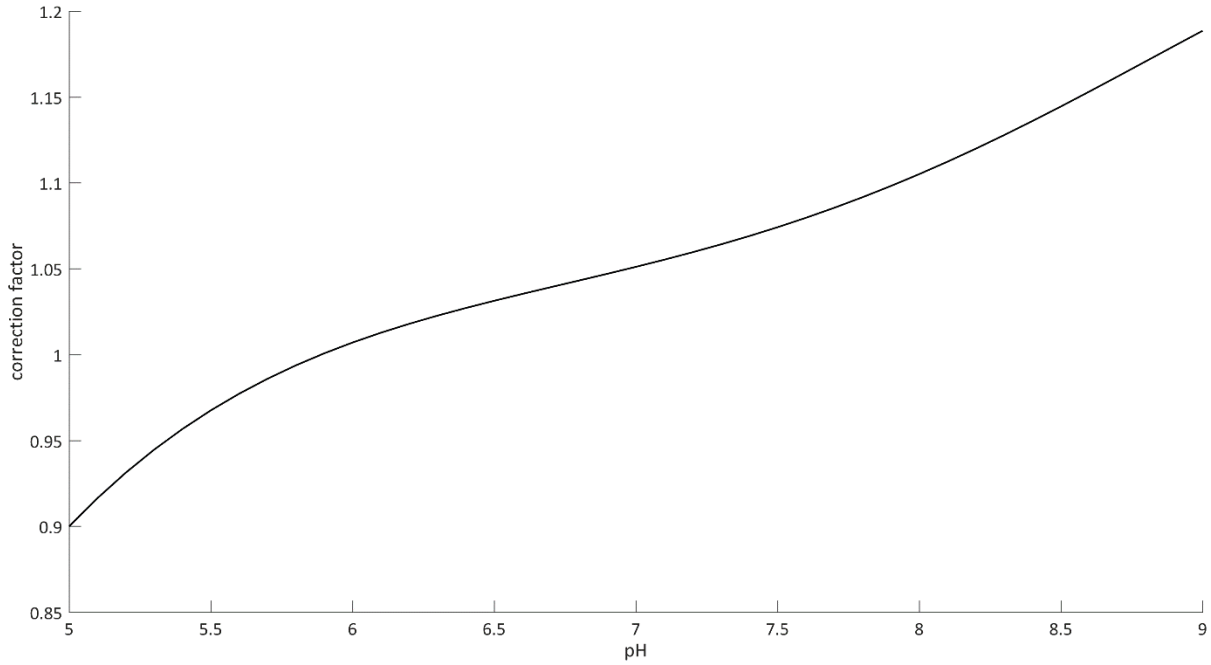

Figure 21: pH correction factor for pH levels from 5 to 9 for Lemna minor

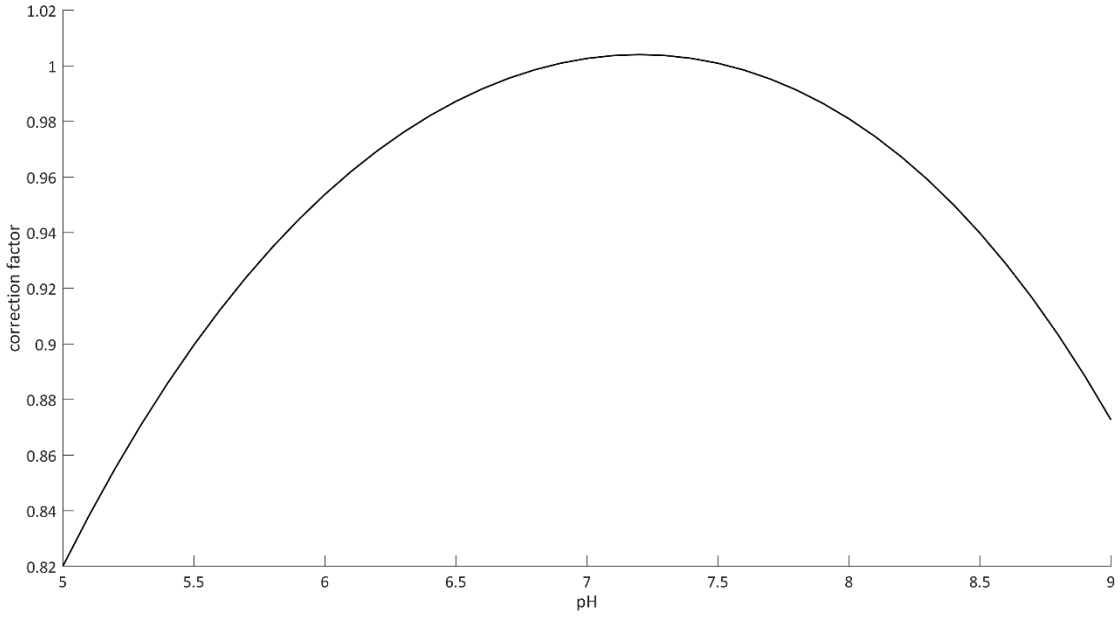

Figure 22: pH correction factor for pH levels from 5 to 9 for Spirodela polyrhiza

Appendix B

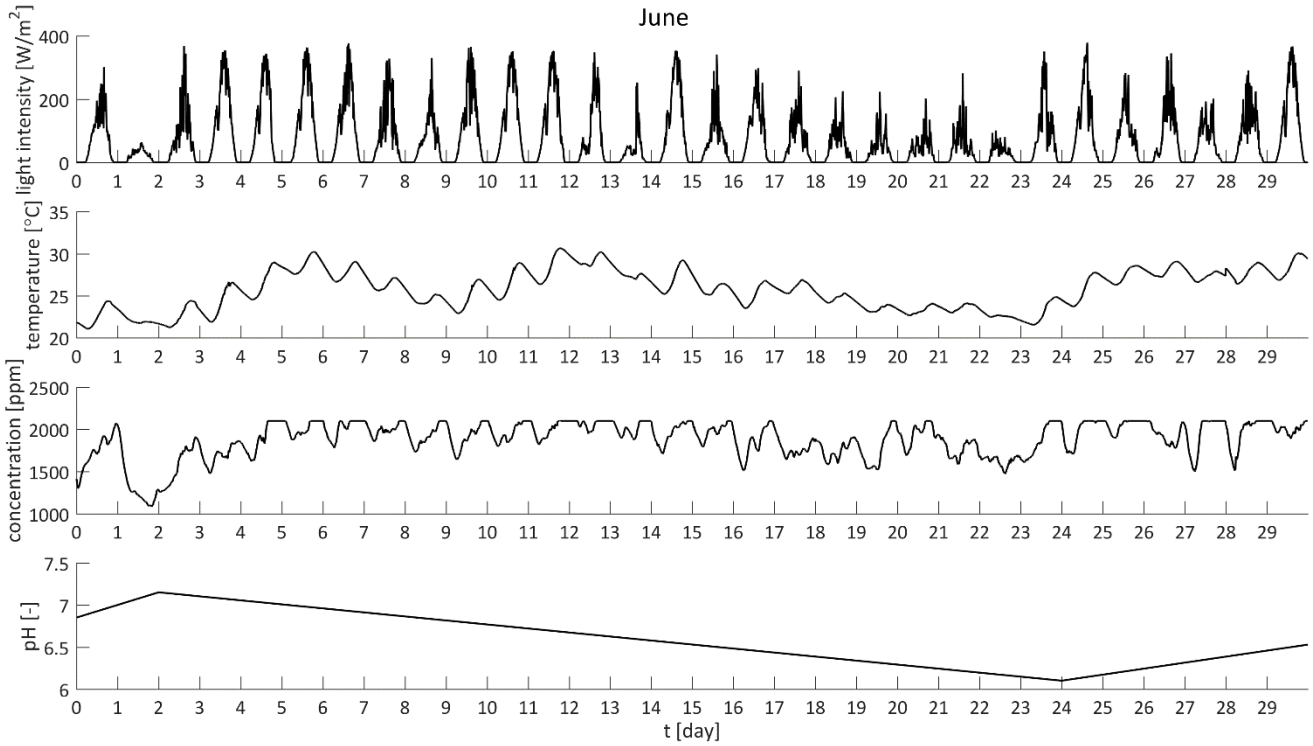

Figure 23: Input data for the month June with light intensity, temperature, CO2 concentration and pH from top to bottom

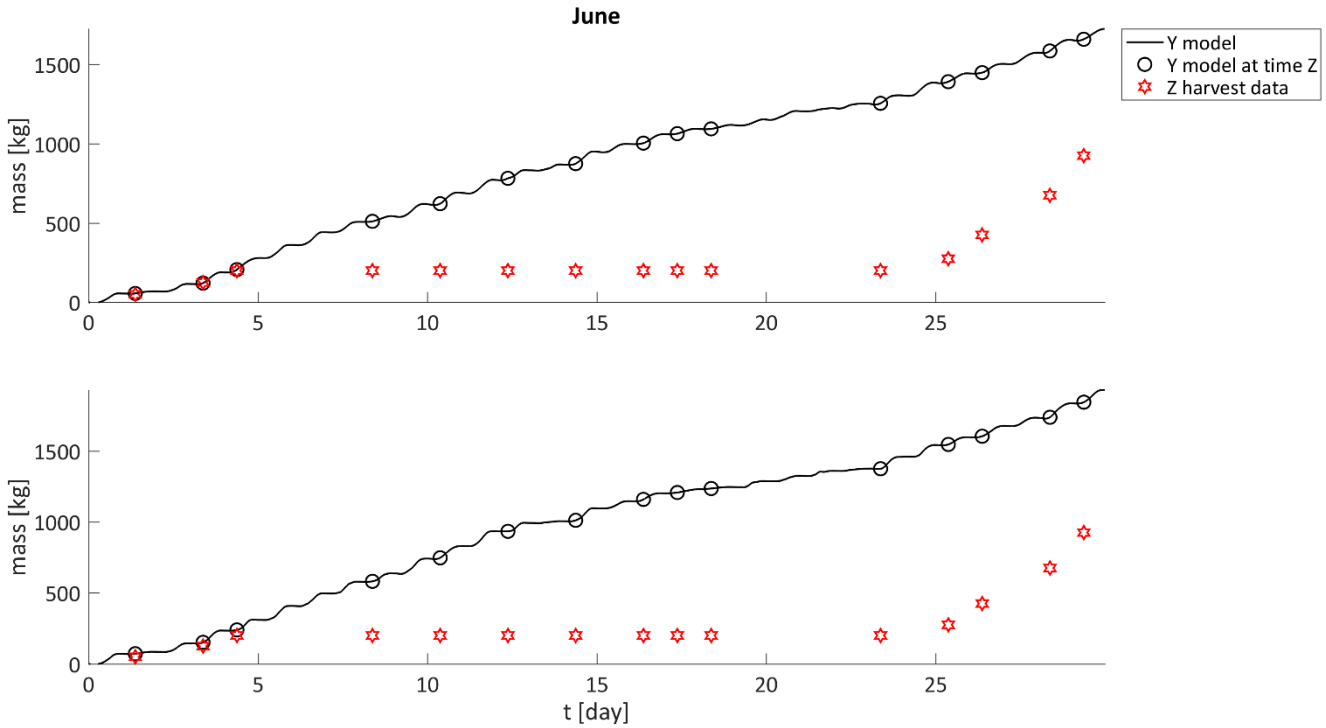

Figure 24: Cumulative production of duckweed at Ecoferm for the month June. The figures describe the model in comparison with the harvest data without and with parameter estimation (above and below respectively)

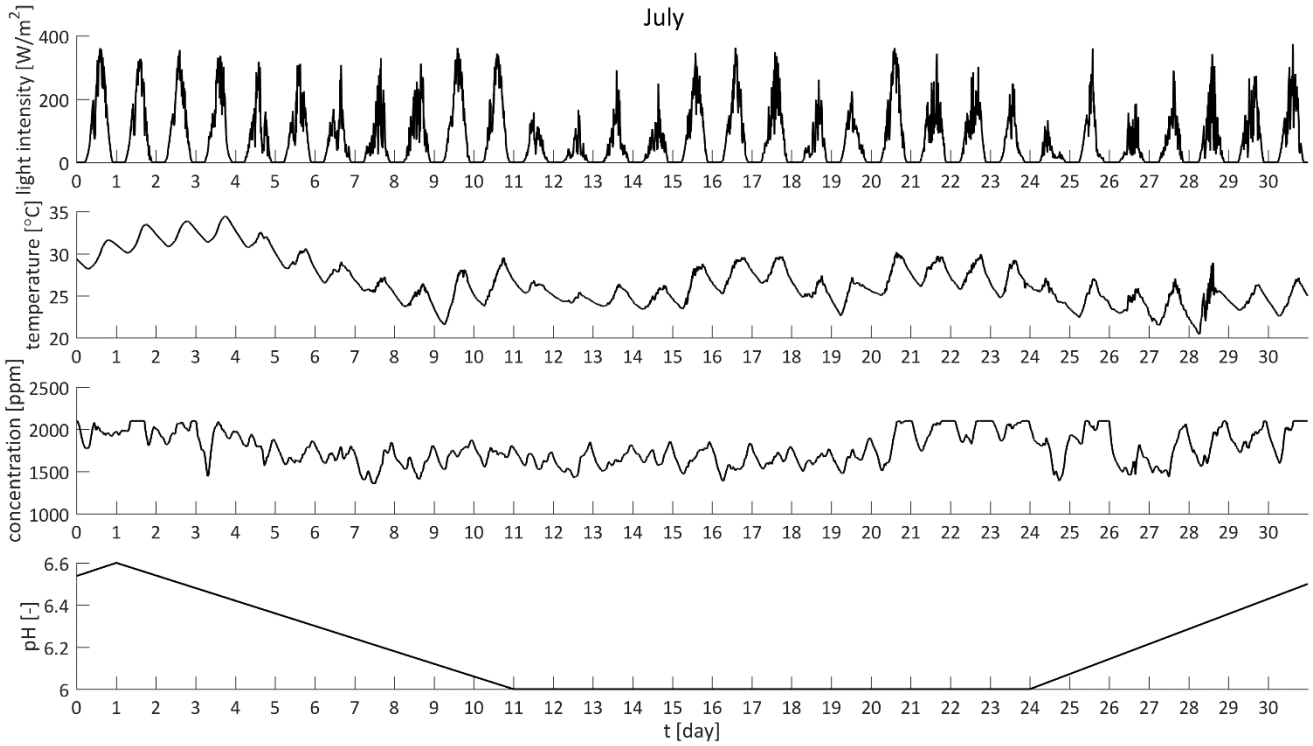

Figure 25: Input data for the month July with light intensity, temperature, CO2 concentration and pH from top to bottom

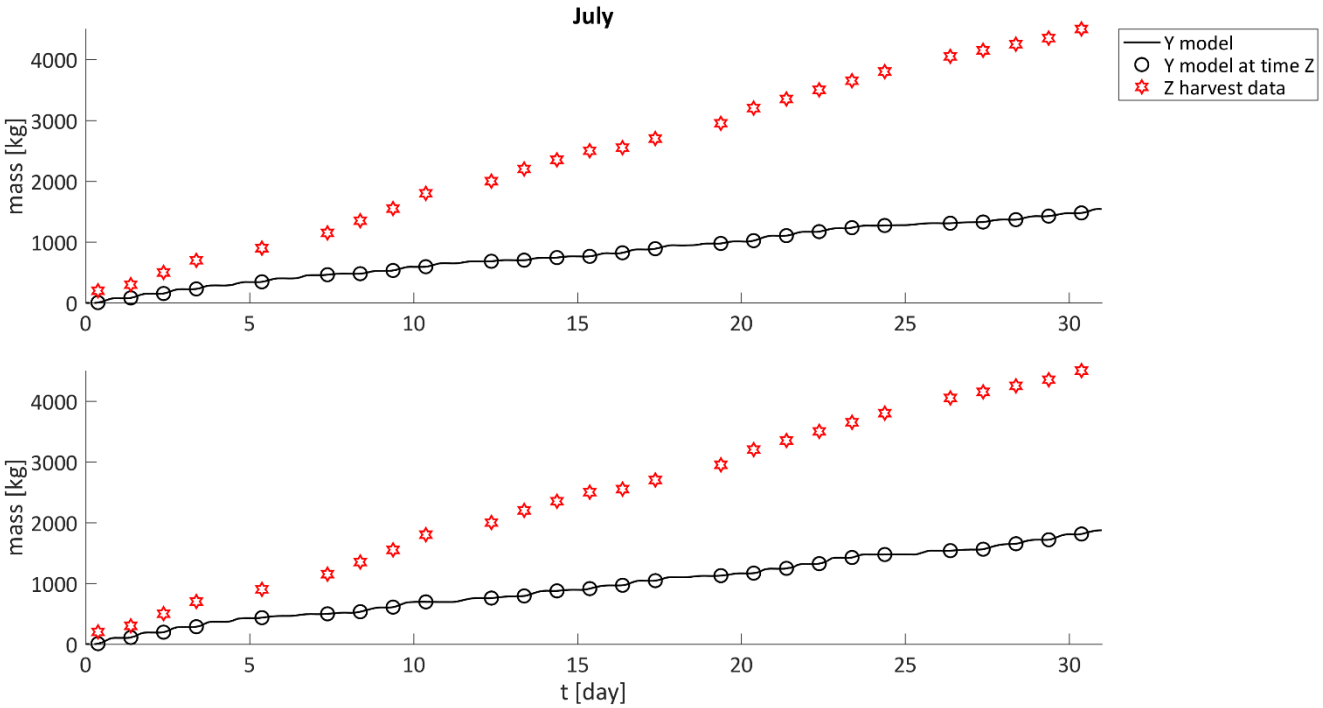

Figure 26: Cumulative production of duckweed at Ecoferm for the month July. The figures describe the model in comparison with the harvest data without and with parameter estimation (above and below respectively)

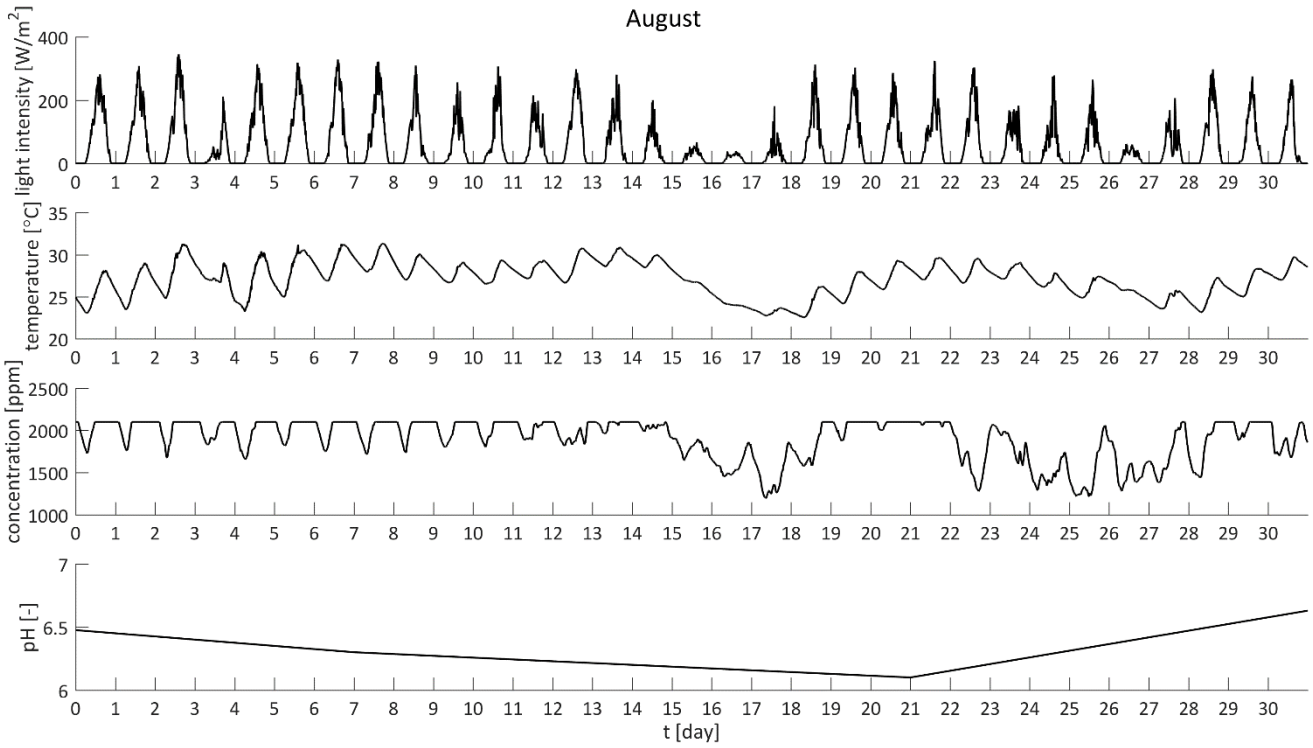

Figure 27: Input data for the month August with light intensity, temperature, CO2 concentration and pH from top to bottom

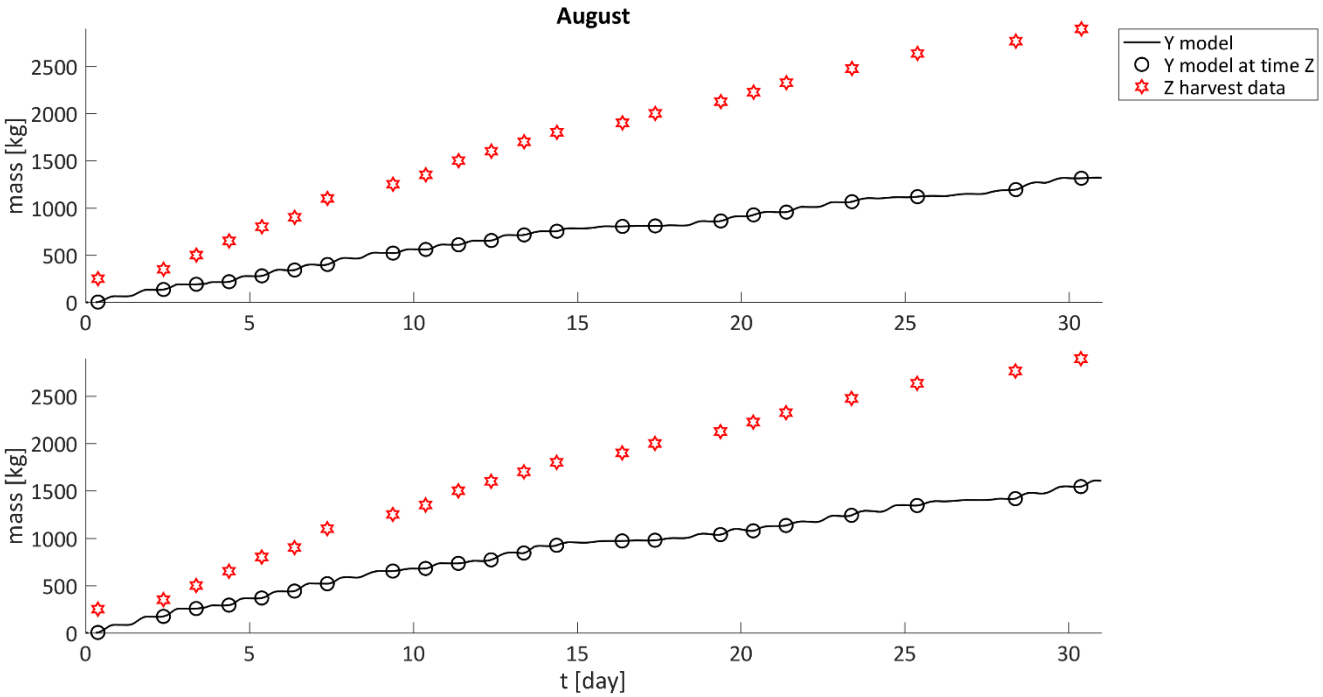

Figure 28: Cumulative production of duckweed at Ecoferm for the month August. The figures describe the model in comparison with the harvest data without and with parameter estimation (above and below respectively)

Appendix C

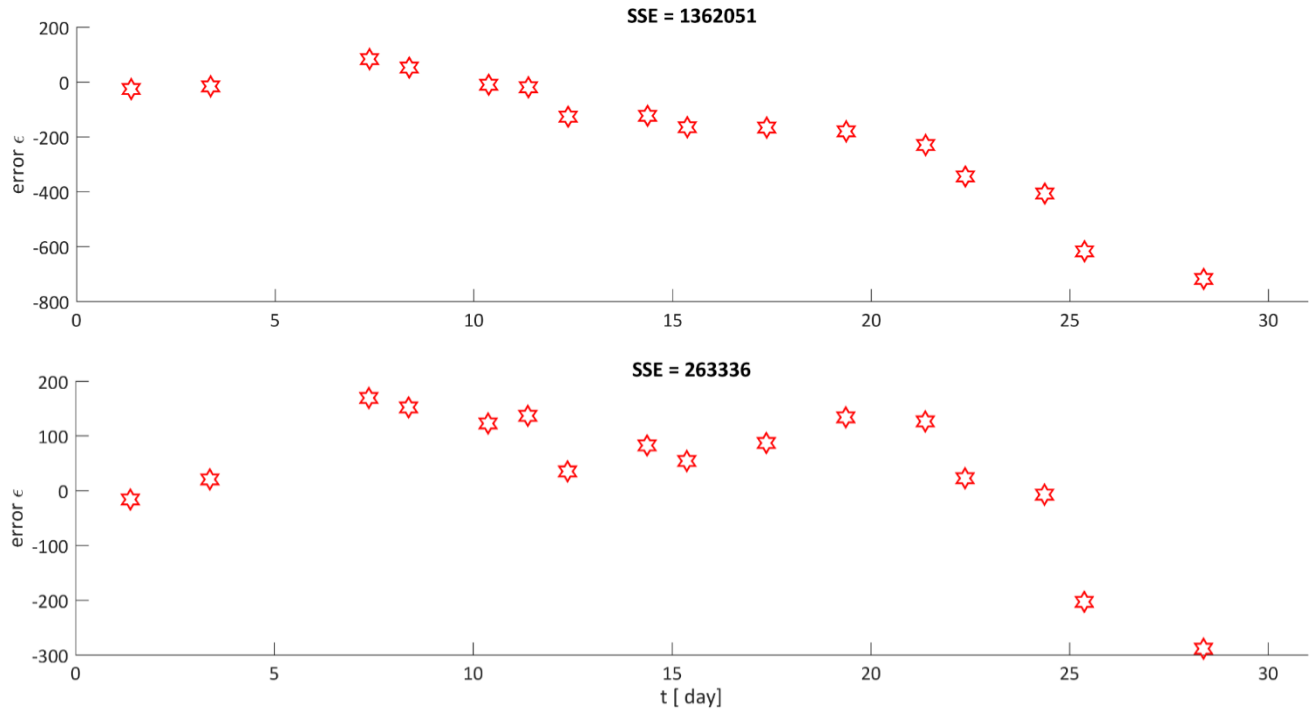

Figure 29: Errors of the cumulative production for the month May (Z-Y)

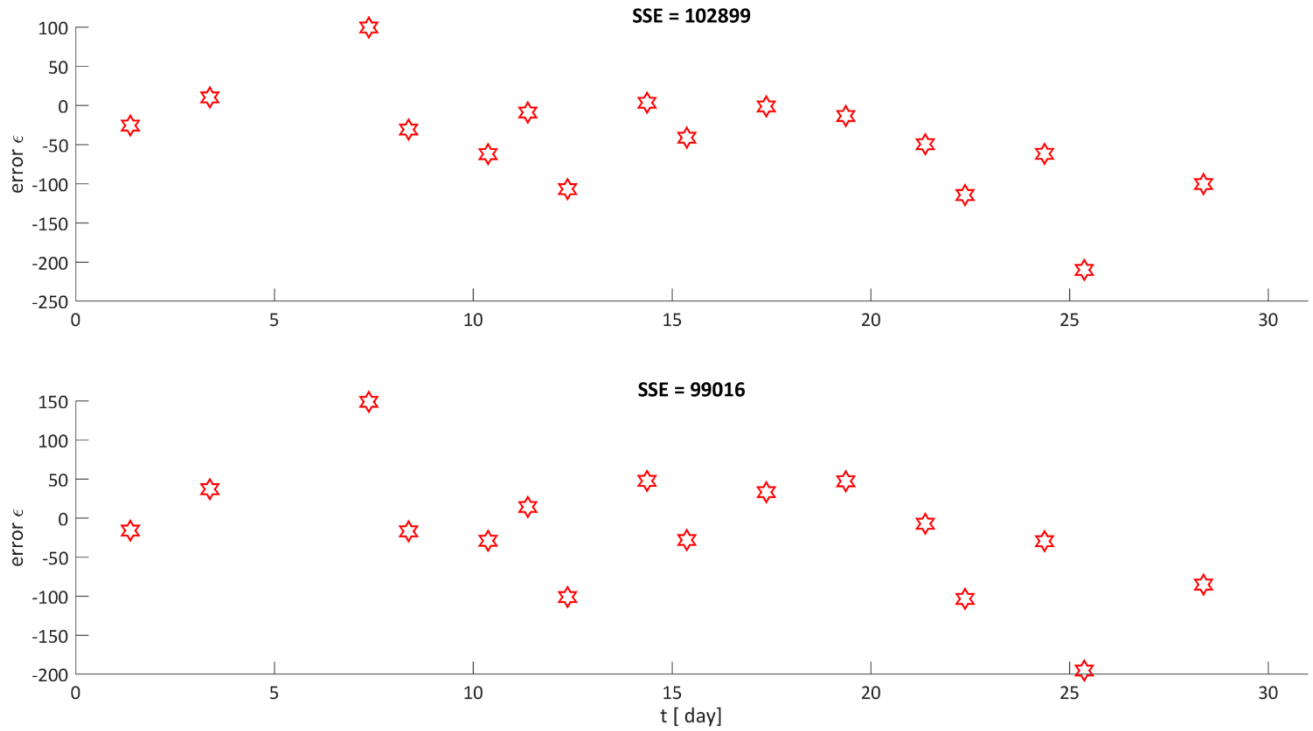

Figure 30: Errors of the daily production for the month May (ΔZ-ΔY)

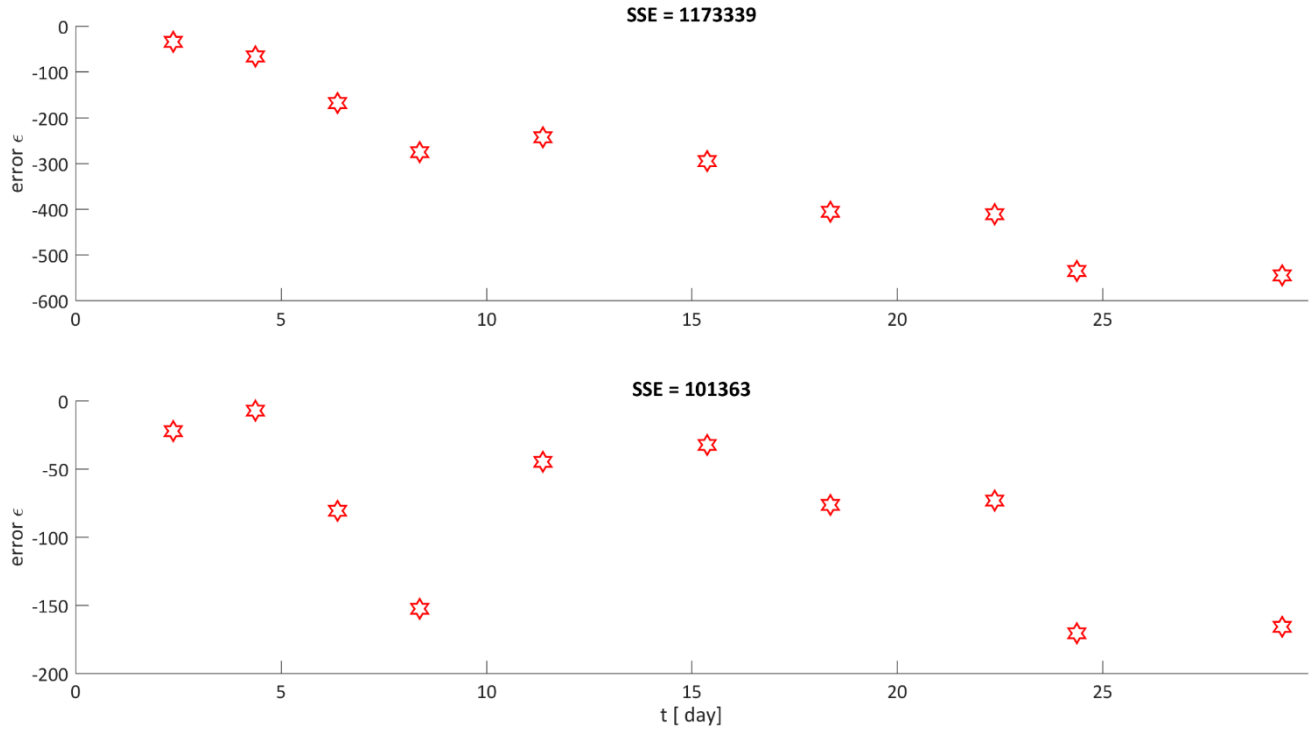

Figure 31: Errors of the cumulative production for the month September (Z-Y)

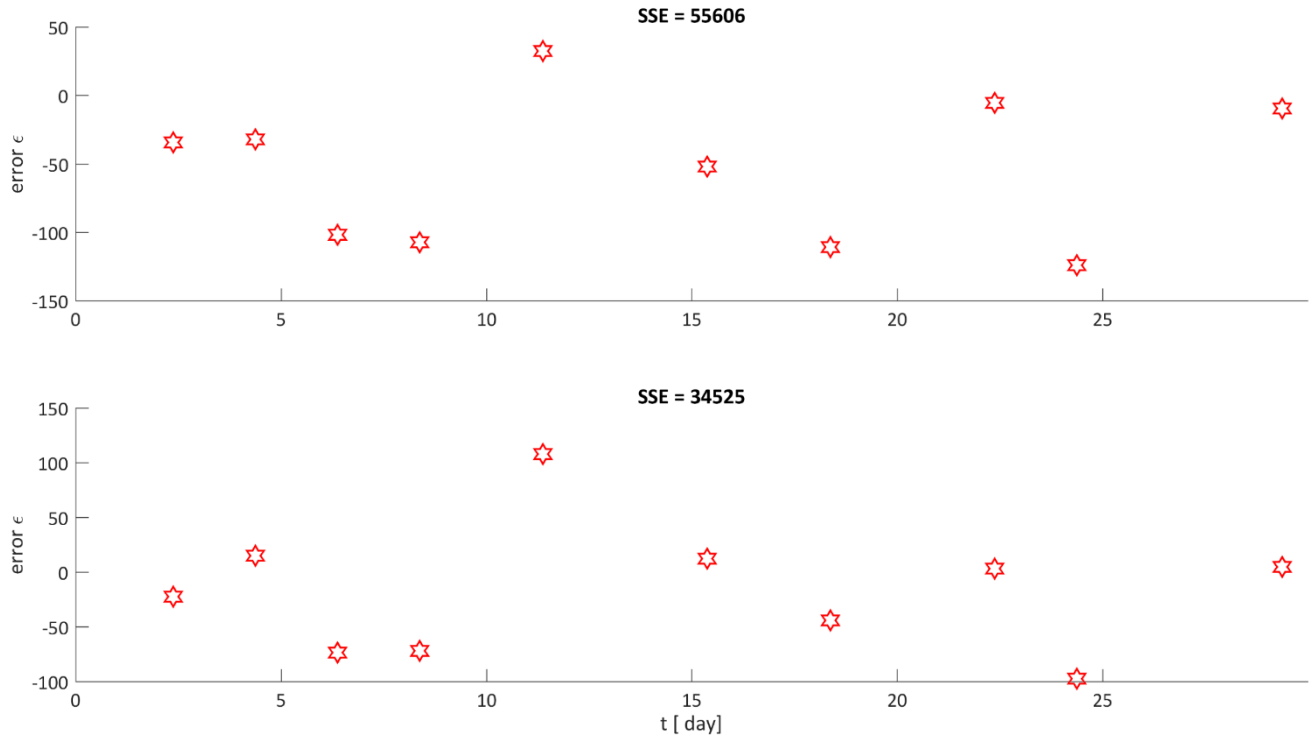

Figure 32: Errors of the daily production for the month September (ΔZ-ΔY)

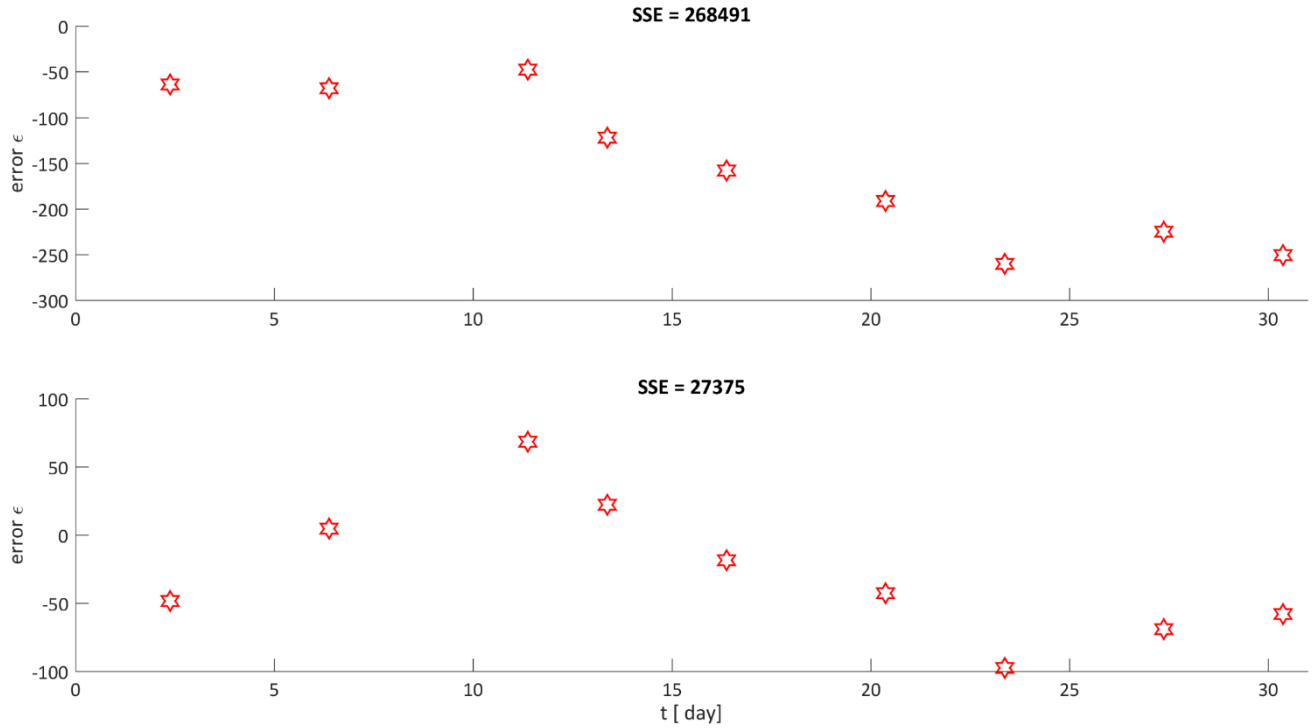

Figure 33: Errors of the cumulative production for the month October (Z-Y)

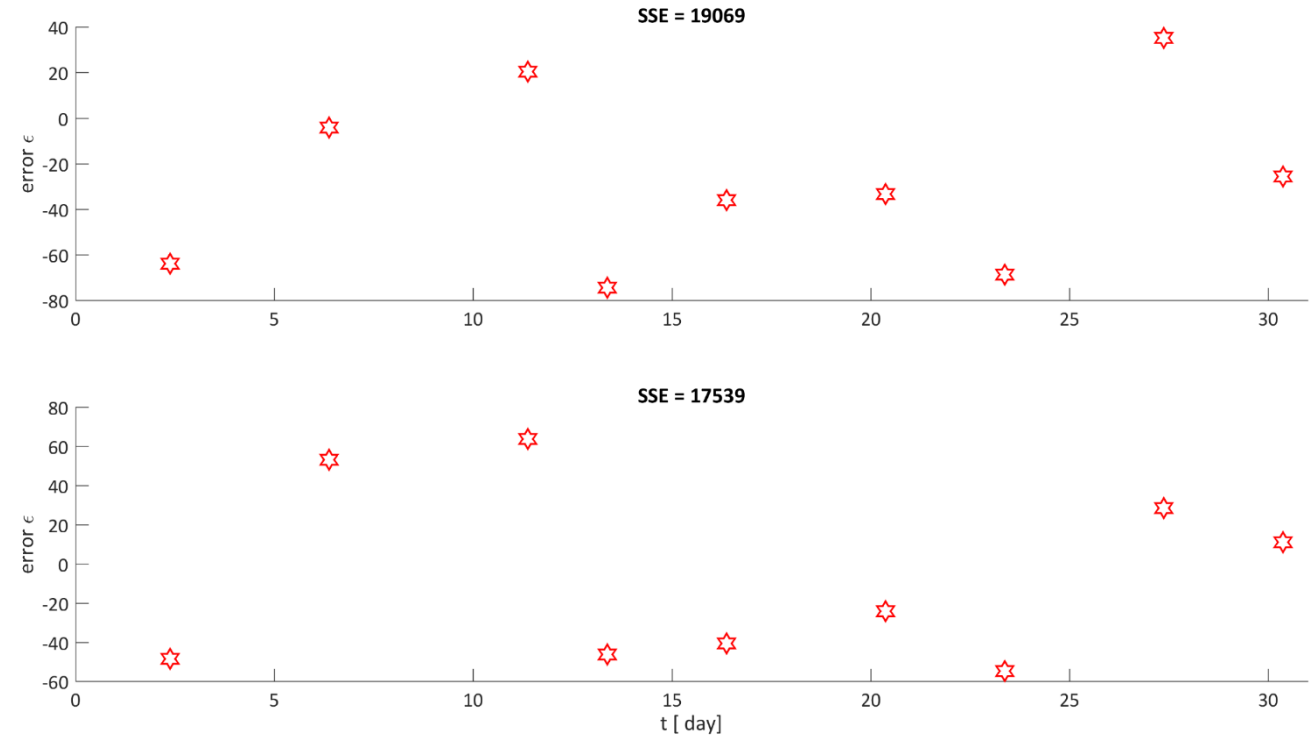

Figure 34: Errors of the daily production for the month October (ΔZ-ΔY)