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Regeneration of Ulva spp. populations in the Wadden Sea after wild harvest

Brandjes, Marvin
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Regeneration of Ulva spp. populations in the Dutch Wadden Sea after wild harvest

Study to determine the influence of wild harvesting *Ulva spp.* populations on regeneration in the Dutch Wadden Sea

Graduation coach:
Wilfred Sewnandan: w.sewnandan@aeres.nl

Student:
Marvin Brandjes: 3022300@aeres.nl

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Abstract

This study has been conducted to estimate the ecological impact of wild harvesting *Ulva spp.* biomass on its population in the Dutch Wadden Sea. The purpose is to give an advice about the ideal time (on the level of population recovery) to wild harvest 1000 kilogram *Ulva spp.* from the Dutch Wadden Sea. The study has been conducted in the context of using seaweed as biomass for human and animal consumption, to solve the issue of lack of food availability. This puts the study in a relevant framework, because of its contribution in sustainable development in food availability. In the Dutch Wadden Sea, not much is known about the possibilities and influence of harvesting wild *Ulva* populations. Besides, at this moment, there is almost no cultivation or wild harvesting present in this area.

This leads to the following main question:

- **<u>What is the impact of wild harvesting *Ulva spp.* biomass on its population in the Dutch Wadden Sea?</u>**

To answer this question, an experiment has been conducted and a simulation model has been used. The experiment was conducted to measure *Ulva* growth in different nitrate- and phosphate concentrations, to find a maximum RGR (relative growth rate). This RGRmax has been used in a simulation model, which has been set up to predict future *Ulva* regrowth, to compare a situation with wild harvesting *Ulva* with a situation without wild harvesting. Besides, the model measures the impact of growth reducing factors (in this study: nitrate, phosphate and temperature). The results show the output of the model, in the form of *Ulva* growth scenarios with different starting biomasses and different temperatures.

The used simulation model is still in development, because the model lacks a couple of crucial growth reducing factors, such as light intensity and mortality.

The main conclusion of the study is that with a higher amount of present biomass and with a temperature closer to the optimum, wild harvesting *Ulva* has less impact on the population. This leads to the following recommendation: for wild harvesting to be environmentally viable, the total biomass in the Dutch Wadden Sea should be high enough, to minimize negative impact. In addition, wild harvesting could be done in summer to ensure a higher RGR.

Nederlandse samenvatting

Dit onderzoek is uitgevoerd om de ecologische impact te schatten van het wildoogsten van *Ulva spp.* biomassa uit de Nederlandse Waddenzee met hierbij het effect op de populatie in dit gebied. Het doel is hierbij om een advies te kunnen geven over de gunstigste tijd (op niveau van populatieherstel) om 1000 kilogram *Ulva spp.* te oogsten uit de Nederlandse Waddenzee. Het onderzoek is uitgevoerd in het kader van het gebruiken van zeewier als biomassa voor consumptie, om op deze manier voedselproblemen te kunnen oplossen (voor zowel mens als dier). Dit zet het onderzoek in een relevant kader, want het draagt bij aan de duurzame ontwikkeling van voedselaanbod. In de Nederlandse Waddenzee wordt tot op heden nog weinig tot niets gedaan aan het verbouwen of wildoogsten van zeewier. Er is hierdoor ook nog niks bekend van de invloed daarvan.

Aan de hand daarvan is een hoofdvraag opgesteld:

- **Wat is de impact van het wildoogsten van *Ulva spp.* biomassa op de *Ulva* populatie in de Nederlandse Waddenzee?**

Om deze vraag te kunnen beantwoorden is een experiment opgezet en een simulatiemodel gebruikt. In het experiment is gekeken naar de invloed van verschillende nitraat- en fosfaatconcentraties op groei van *Ulva* uit de Waddenzee, om zo tot een maximale RGR (relatieve groeisnelheid) te komen. Deze RGRmax is gebruikt in het simulatiemodel, opgezet om een voorspelling te doen over toekomstige *Ulva* aangroei, om een situatie met uitvoering van wildoogsten te vergelijken met een situatie zonder wildoogsten. Daarnaast meet het model de impact van verschillende groei-reducerende factoren (nitraataanbod, fosfaataanbod, watertemperatuur). In de resultaten is de output van het model weergeven, in de vorm van een aantal *Ulva* groeiscenario's, met verschillende startbiomassa's en verschillende temperaturen (10˚C, 15˚C, 20˚C).

Het simulatiemodel bevat nog een aantal gebreken. Dit zit vooral in het ontbreken van cruciale groei-reducerende factoren, als lichtintensiteit en mortaliteit.

De belangrijkste conclusie van dit onderzoek (antwoord op onderzoeksvraag) is dat wanneer de aanwezige *Ulva* biomassa hoger wordt en de watertemperatuur dichter bij *Ulva*'s optimum ligt, wildoogsten een lagere impact heeft op de populatie. Dit leidt tot de volgende aanbeveling: Om het wildoogsten van *Ulva* uit de Nederlandse Waddenzee ecologisch haalbaar te maken, dient het wildoogsten te gebeuren met een hoge aanwezige biomassa, om de kans van negatieve impact op de populatie te verkleinen. Daarnaast is het handig om het wildoogsten in de zomer plaats te laten vinden, omdat *Ulva* dan een hogere RGR heeft.

Contents

1. Introduction

Worldwide, there is a growing demand for sustainable products, to be used as both food (human consumption) and animal feed additives. Current land-based agriculture is not able to provide enough biomass for these purposes, mainly because of an increasing world population. An increasing amount of land is needed, due to demand of agricultural space to provide today's world population with biomass for consumption. (Macedo *et al*., 2012). Researchers worldwide are looking for innovative ways to be able to provide more biomass for consumption.

Seaweed harvesting and cultivation could be a solution for this problem (Loureiro *et al*., 2015). The majority of commercially scaled seaweed (99% in 2012) is produced in Asian countries, especially in countries as China (54%), Indonesia (28%), the Philippines (7%) and North and South Korea (4%) (Taelman *et al*., 2015). This dominance is mainly due to the long history of human consumption of a wide variety of seaweeds, such as *Pyropia spp., Porphyra spp., Laminaria spp., Saccharina spp.* and *Undaria pinnatifida.* European import of seaweed are traditionally used by the pharmaceutical, cosmetic and food industry for its useful extracts (e.g. phycocolloids such as agar) or as products for agriculture (fertilizer, cattle feed, fish feed) and are less commonly used for direct human consumption. Compared to Asia, European seaweed production is still relatively small in scale and is mostly found in countries such as France, Spain, Portugal, Ireland and Norway, either as commercial or experimental setups. The main European cultivated species to date are *Saccharina latissima* (sugar kelp) and *Undaria pinnatifida* (Wakame), and there is an increasing interest in *Ulva spp*., mostly for its fast growth rate (more biomass over time). (Taelman *et al*., 2015 ; Van den Burg *et al*., 2013). Due to the high population density in Western Europe, there is great competition for land, arising from the growing demand for food, energy and accommodation. Therefore, seaweed cultivation and wild harvesting in European seas could be a solution to reduce the agricultural pressure on land and its resources. (Taelman *et al*., 2015).

Wild harvesting seaweeds from Europe's seas and oceans may seem like an easy and economically viable option, there are several issues to deal with using this method of harvesting. The biggest issue would be the environmental viability; the possibility of stock depletion of the wild population, which could lead to both environmental and ecological impacts in the area the seaweed has been taken from. (Mac Monagail *et al*., 2017). To prevent stock depletion, calculations and predictions on regeneration have to be made before the actual wild harvesting. Besides stock depletion, the act of wild harvesting seaweed could also lead to environmental issues, such as soil disturbance, which is a major problem in the Dutch Wadden Sea right now. This study only focuses on the impact of wild harvesting *Ulva spp.* on its regeneration in the Dutch Wadden Sea, which will be calculated by using a self-made simulation model. This model serves the purpose to be able to estimate *Ulva* production under a range of nitrate/phosphate concentrations and water temperatures.

The purpose of this study is to emphasize the importance and possibilities of sustainable agriculture in the Dutch Wadden Sea. The study mostly applies to European development in sustainability of agriculture. The FAO also emphasizes the importance of using seaweed instead of land-based agriculture, due to overexploitation. The article also implies that seaweed becomes more important in IMTA-systems (Integrated multi-trophic aquaculture), especially in countries like Brazil, China and the Republic of Korea, due to their wide sea-agricultural use. (Ferdouse *et al*., 2018). This puts the importance of sustainable sea agriculture on a worldwide scale. In European context, the results of this study could be important for Universities or Marine Research Institutions, such as Wageningen Marine Research. Besides that, agricultural Universities (for example Wageningen University) are already conducting research to the possibilities of using seaweed as substitute for land-based agriculture. This subject also applies to anyone interested in further developments in using seaweed as human food or animal feed, and the expansion of seaweed harvest and cultivation in areas where there is (almost) no present harvest or cultivation, such as the Dutch Wadden Sea.

1.1 Area description

The area used in this study is the Dutch Wadden Sea. This area is chosen, because at this moment, there is almost no seaweed cultivation or wild harvesting present. However, according to fishermen in this area, there is a high abundance of *Ulva* present. This observation is based on the huge amounts of *Ulva* stuck in their fishing nets during summer time (figure 1) (Jak, 2019). The actual presence of *Ulva* in the Dutch Wadden Sea in kilograms is unknown. For this reason, the abundancy of *Ulva* is merely based on observations. This leads to hypothetical scenarios in the simulation model. But, in the near future, Wageningen Marine Research is planning to study the abundance of *Ulva* in the Dutch Wadden Sea (Jak, 2019). These studied values could be used in the simulation model to simulate the actual field values.

Figure 1: Ulva in fishing nets in the Dutch Wadden Sea

The high abundance of *Ulva* could also be expected by the present biotic and abiotic values in the Dutch Wadden Sea. These values are suitable for *Ulva* growth. For example, nutrient influx (nitrate, phosphate) is highly present, mainly from the Ijsselmeer and the river Eems. Besides that, there's also an in-outflow of nutrients between the North Sea and the Dutch Wadden Sea (figure 2). The inflow of nutrients from the North Sea to the Wadden Sea is mainly in coastal flows, which contains water from the great rivers (Rhine, Noordzeekanaal etc.) (Kloepper *et al*., 2017). The nutrients are distributed throughout the Wadden Sea by the current flow, shown in figure 3. This influx could have a positive effect on the *Ulva* presence. (Kloepper *et al*., 2017). Additionally, the Dutch Wadden Sea still contains a high nutrient level, due to nutrient leaching of agricultural fertilization (Kloepper *et al*., 2017).

The Dutch Wadden Sea is part of the Natura 2000 network, which means that the entire area is protected by the European law system. In this context, the act of wild harvesting *Ulva spp.* from the area needs an exemption by law. This means, in order to wild harvest huge amounts of seaweed, cooperation with the government is necessary. (Bij12, 2019).

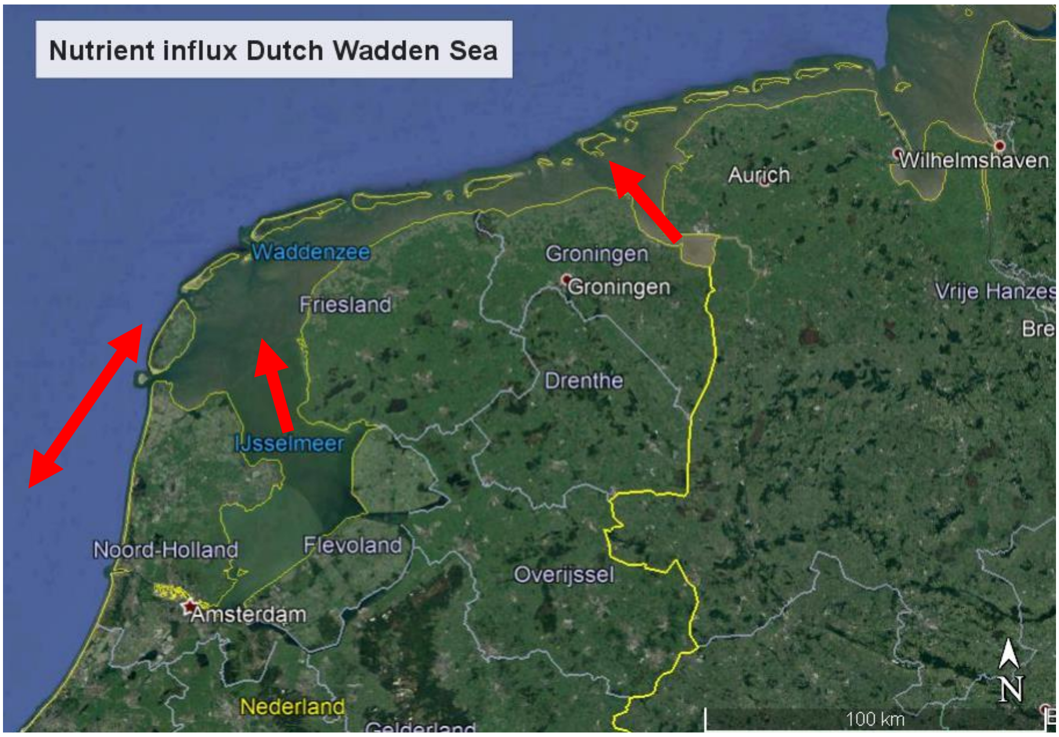

Figure 2: Nutrient influx Dutch Wadden Sea

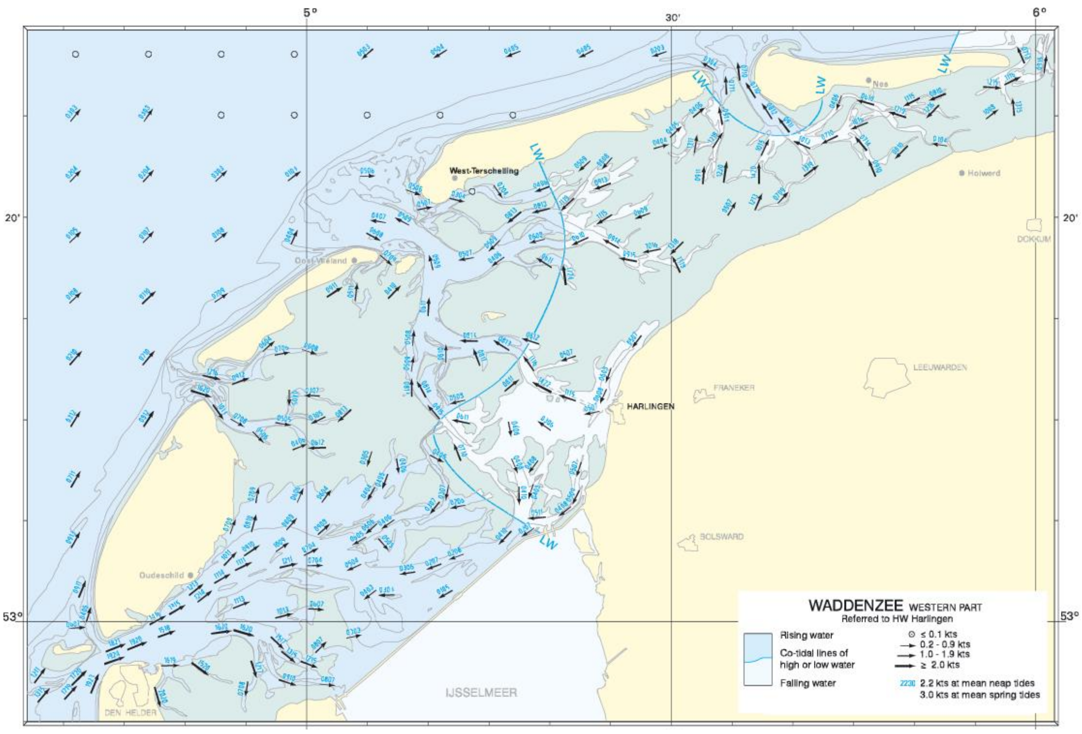

Figure 3: Current direction Dutch Wadden Sea

1.2. *Ulva spp.*

Ulva is a green algae genus in the family of *Ulvaceae*. In the common tongue *Ulva* is known as sea lettuce, which is given for its green lettuce-like appearance. *Ulva* organisms start as free-floating single-celled duplicating organisms, until the seaweed anchors itself to solid objects, such as shells, rocks and other materials, where its further growing process can start. In its wild habitat, *Ulva* can reach a maximum length of 30 centimetres and has a high maximum relative growth rate (RGRmax) of approximately 0.31 (31%) per day, under optimal growing circumstances (Breure, 2014). Because of this high RGR, *Ulva* populations can regrow relatively fast, and this could be an environmentally viable option for wild harvest. The colour of *Ulva* leafs is depending on the availability of nitrogen. The more uptake of nitrogen, the greener the leaves (Riccardi, 1996). *Ulva* grows in warmer waters (10-25 °C) and is a translucent plant (Breure, 2014). According to Gao (2016), the optimal nutrient concentration in the water is 150 µM nitrogen (N) and 7.5 µM phosphorus (P), with a water temperature of 18-20 °C.

In most scientific reports, the most commonly used *Ulva* species is *Ulva lactuca* (figure 4). However, research shows that in older reports every used species was called *Ulva lactuca*, while it turned out to be another species (Stegenga & Mol, 2002). In Dutch waters, there are more *Ulva spp.* present, apart from *Ulva lactuca*, namely: *Ulva pertusa (*Figure 5)*, Ulva rigida, Ulva curvata, Ulva pseudocurvata* and *Ulva tenera* (Jak, 2019 ; Stegenga & Mol, 2002). Despite the presence of multiple species, the growing conditions of these different *Ulva* in the Netherlands are quite comparable, so the optimal conditions of *Ulva lactuca* have been used in this experiment as a baseline.

Figure 4: Ulva lactuca

Figure 5: Ulva pertusa

1.3. Abiotic factors

Ulva spp. in the Dutch Wadden Sea are influenced by abiotic factors. In the simulation model used in this study, temperature, nitrogen and phosphorus are included. Besides these values, there are other crucial influential factors, such as light availability, mortality and salinity. The light availability and salinity are not included in this study due to the usage of average values. For light availability, this value has been stated at 12 hours of light per day (average during spring/summer). The salinity of the Wadden Sea is approximately 28 g/L, while *Ulva* has an optimum curve for salinity from 15 g/L until 63 g/L. For this reason salinity will not be included in this study. Mortality is not included in this study, because the model is based on unlimited growth.

1.3.1. Temperature

Most seaweed species have a minimum and a maximum water temperature to be able to exist (Stichting Noordzeeboerderij, 2017). *Ulva lactuca* for example has an optimal temperature scale from 15°C till 25°C (Groenendijk *et al.,* 2016), with the optimum around 18-20°C (Gao, 2016). When the water temperature becomes too low, *Ulva* will grow a lot slower, or even stay in a rest phase, not growing at all. This rest phase will occur until a threshold of approximately 5°C. Below this temperature, the seaweed is likely to die (Groenendijk *et al.,* 2016). When the temperature becomes too high, *Ulva* will most likely die. For example, when the temperature increases above the maximum survival temperature of the crop, a phenomenon called ´temperature shock' will occur. The membranes of the cells will become too fluid, leading to total leakage of the cells, including gametophytes and chlorophytes (Fan *et al.,* 2017) (figure 6). Additionally, the heat can lead to inefficient respiration and denaturation of proteins (Van 't Klooster, 2018). This could also happen when the *Ulva* is taken from a rather cold environment and placed into a warmer environment (Fan *et al.,* 2017). For example, by taking *Ulva* leafs from seawater which is 5°C and putting it in seawater which is 20°C. Temperature is a seasonal factor and will be used in the model as reducing growth factor, with an optimal growing temperature of 18°C (Gao*,* 2016). The average weekly temperature of the seawater, which is used in the simulation model, can be deduced from temperature maps saved in a data base from the Royal Netherlands Meteorological Institute (KNMI, 2019).

Figure 6: Ulva spp. cellular leakage (observation during this study)

1.3.2. Nutrients

Seaweed species need, like all plants, nutrients to grow. The most important nutrients for seaweeds are nitrogen and phosphate, since these nutrients are usually present in relatively low concentrations and therefore potentially control the growth rate (Kloepper *et al*., 2017). However, this does not apply to the Wadden Sea, due to its high nitrogen concentration (as stated in 1.1. Area description). The actual nitrate concentration in the Dutch Wadden Sea is 50 µM (Kloepper *et al*., 2017). This value is quite high, as the nitrate uptake by *Ulva* in this concentration is 6 µM, which mean there is plenty nitrogen available in the Dutch Wadden Sea and it is not a huge reducing factor on *Ulva* growth (Breure, 2014). The actual phosphate concentration is 1 µM (Kloepper *et al*., 2017) and the uptake by *Ulva* is 1.276 µM. (Breure, 2014). This could be a problem in *Ulva* growth, noting the phosphate uptake is greater than the availability (Breure, 2014). This makes the reducing factor phosphate a limiting factor of *Ulva* growth in the Dutch Wadden Sea. These are used as influential factors in the simulation model. (Breure, 2014 ; Kloepper *et al*., 2017). Despite of this high nutrient level, particularly during the spring bloom, phytoplankton (including *Ulva*) may experience P-limitation (Jung *et al*., 2017). The further away from the shore, the less nutrients there are available. The availability of nutrients and uptake of nutrients by seaweeds is also determined by the water flow in the growing area (Thieltges, 2017) (see figure 2 and 3 in 1.1. Area description). In the Dutch Wadden Sea, the distribution of nutrients is quite fast, due to the relatively small surface. (Thieltges, 2017 ; Kloepper *et al.*, 2017). This could mean a massive growth of *Ulva* biomass, throughout the entire Wadden Sea. (Van 't Klooster, 2018). Besides that, there is an interaction between water temperature and nutrients. The nutrient availability strongly modulates the thermo-tolerance of *Ulva* in seawater (Van 't Klooster, 2018).

Mentioned abiotic factors influence the growth of wild *Ulva* populations. Whereas water temperature can be deducted from sea climate maps. Nitrate and phosphate values can be deducted from articles. However, to get the maximum relative growth rate in optimum circumstances, laboratorial experiments have to be conducted. These abiotic reducing values are to be used in a model to reduce the maximum relative growth rate, to consequently be able to simulate *Ulva* growth in order to calculate the influence of wild harvesting *Ulva* on the wild population.

In the Netherlands, most wild harvesting, cultivation and research on seaweed occurs in the North Sea and the Eastern Scheldt (for example Noordzeeboerderij, 2017 ; Seaweed Harvest Holland, 2015). The possibilities of wild harvesting seaweed from the North Sea are proven to be both environmentally and economically viable (Mac Monagail, 2017). However, in the Dutch Wadden Sea, not much is known about the possibilities and influence of harvesting wild *Ulva* populations. This includes the possibility of stock depletion. Added to that, fishermen in the Dutch Wadden Sea are complaining about the huge amounts of *Ulva spp.* stuck in their nets during summer (1.1. Area description, figure 1), which leads to an opportunity to make wild harvesting viable on a social level. Research will be conducted in order to predict the impact of wild harvesting *Ulva* biomass from the Dutch Wadden Sea, in the context of environmental viability of the regeneration of *Ulva*.

Main question:

- **When has wild harvesting *Ulva spp.* biomass the least impact on its population in the Dutch Wadden Sea?**

The main question will be answered by using a simulation model (2.3.), supplemented with experimented values, in which different scenarios will be applied. This will provide an answer for the following subquestions:

- **What is the maximum relative growth rate (RGR) of Ulva grown in which nitrate and phosphate concentrations?**
- **What is the difference in *Ulva* regrowth after 1000kg wild harvest, comparing to a situation where there is no wild harvest, according to the used simulation model?**
- **What is the difference in biomass output, using different seasonal temperatures (10˚C, 15˚C, 20˚C)?**
- **Which reducing factors have the most influence on the relative growth rate (RGR) of *Ulva* in the Dutch Wadden Sea?**

The expectation is that *Ulva* populations will regrow relatively fast (due to its high relative growth rate), provided that wild harvesting 1000 kg/day doesn't deplete the entire stock in the Dutch Wadden Sea (for now based on observations / random choice, see 1.1. Area description), and thus be environmentally viable. This 1000kg harvest per day could be used for animal or human consumption.

Reading guide:

At the start of this thesis, two summaries can be found: a Dutch and English summary. After that, chapter 1 describes the introduction, followed by the materials & method in chapter 2. Chapter 3 presents the results, which is interpreted in chapters 4 and 5: discussion and conclusion. Afterwards, the acknowledgements, bibliography and appendices are shown.

2. Materials & Method

In context of this study, a method was developed to measure growth of *Ulva* in different nutrient concentrations (nitrate, phosphate). Besides this, a literature study has to be conducted in order to know the influence of these concentrations on *Ulva*. The consequential RGRmax (maximum relative growth rate) value from these experiments will be used in a simulation model for *Ulva* growth in the Wadden Sea. In order to conduct the developed growth experiments, *Ulva* needs to be collected from the field.

2.1 Field collection

Ulva samples have to be taken from wild populations in the Dutch Wadden Sea. All used samples are taken from tidal influenced rocks, located on the pier next to the TESO Haven in Den Helder. Enough *Ulva* has to be taken to cut out at least 100 circles of 1cm diameter. Directly after the field sampling, the collected *Ulva* will be processed in the laboratory. Further information on this matter is explained in 2.2 Growth Experiments. The wild samples need to be taken based on size and completeness, as in *Ulva* individuals with the least amount of holes in its leaf area.

2.2 Growth experiments

Growth experiments were performed based on slices of Ulva (1 cm diameter) were taken from Ulva collected from the field. These were incubated in Erlenmeyer flasks with adjusted F2-medium (2.2.1. and appendix I) and varying nutrient concentrations, and the increase in biomass is followed by weighting the slices during approximately 2 weeks (until t = 14). The relative growth rate was then estimated and plotted into graphs. This process is explained in the following paragraphs.

2.2.1. Nutrient concentrations

Laboratorial research has to be conducted in order to find the relative growth rate (RGR) of *Ulva spp.* in different growing conditions, e.g. different nitrogen and phosphorus concentrations in the growing medium (F-2 medium). For this study, adjusted F2-medium was used to grow *Ulva*. Normal F-2 medium contains an excess of the amount of all substances needed for normal growth.

To start off, adjusted F-2 medium has to be prepared. This medium consists of 2 µm 30‰ filtered seawater (FSW) supplemented with $NaNO_3$, $NaPO_4$, trace elements (Appendix I) and vitamins (Appendix I). The used amounts of $NaNO_3$ and $NaPO_4$ are summed up in table 1. Trace elements and vitamins have to be added in the concentration 1 millilitre (1000 µL) per litre medium (Guillard & Ryther, 1962 ; Guillard, 1975). This adjusted medium is put in amounts of 150 ml into 9 Erlenmeyer flasks with different $NaNO_3$ or $NaPO_4$ concentrations (Tables 1 and 2). These values were selected by doubling and halving the optimal nitrogen and phosphorus values for growth of *Ulva rigida*, according to research of Gao, 2016. This is done to be able to find the maximum RGR of the *Ulva* samples from the Wadden Sea.

Table 1: Experiment with different NaNO3 concentrations

Erlenmeyer	$NaNO_3$ (µM/L)	$NaPO_4$ (µM/L)	Trace elements (µl/L) (µM in Appendix 1)	Vitamins (µl/L) (µM in Appendix 1)
1	9.375	7.5	1000	1000
2	18.75	7.5	1000	1000
3	37.5	7.5	1000	1000
4	75	7.5	1000	1000
5 (Optimum according to Gao, 2016)	150	7.5	1000	1000
6	300	7.5	1000	1000
7	600	7.5	1000	1000
8	1200	7.5	1000	1000
9	2400	7.5	1000	1000

Table 2: Experiment with different NaPO4 concentrations

Erlenmeyer	$NaPO_4$ (µM/L)	$NaNO_3$ (µM/L)	Trace elements (µl/L) (µM in Appendix 1)	Vitamins (µl/L) (µM in Appendix 1)
1	0.46875	150	1000	1000
2	0.9375	150	1000	1000
3	1.875	150	1000	1000
4	3.75	150	1000	1000
5 (Optimum according to Gao, 2016)	7.5	150	1000	1000
6	15	150	1000	1000
7	30	150	1000	1000
8	60	150	1000	1000
9	120	150	1000	1000

2.2.2. Growth incubations

Subsequently, 45 slices of approximately 1 cm diameter have to be cut out of multiple harvested wild *Ulva* thalli. This can be done using different tools, as long as it's sharp and round. These *Ulva* slices will be weighed (fresh weight) by dipping the pieces on filter paper, to subsequently put on a scale (further weighing information in next paragraph). Afterwards, the slices are randomly put into 9 Erlenmeyer flasks with 150 ml adjusted F-2 medium with different nitrogen or phosphorus concentrations (see above), whereas every flask contains 5 different slices of *Ulva*. After weighting and distributing the *Ulva* slices, the flasks will be put on a rocking platform in a climate chamber, using 10 °C as the temperature value and preferably 6000 Lux as light intensity. In the experiments of this study, the light intensity is set at approximately 5800 Lux.

Every 2, 3, or 4 days, the *Ulva* slices are filtered out of the different flasks and weighted again, until approximately day 15. This weighting is done by dabbing the *Ulva* disks on a tissue. Afterwards, the disks are put on a scale and the weight is noted with 4 decimal points. The emptied flasks are refilled with fresh F-2 medium with the different concentrations of $NaNO_3$ or $NaPO_4$, the *Ulva* disks will be put back into these refilled flasks and the flasks will be put back in the climate chamber.

2.2.3. Relative Growth Rate (RGR)

Data will be implemented into Excel, so scatterplots can be made per flask to assess the relative growth rate (RGR), calculated by using the following formula: RGR = 100*(ln(W2)-ln(W1))/(T2-T1) (Wu et al., 2018). Besides using this formula, there is another way to generate the RGR of the different Erlenmeyer flasks. This is done by drawing a trend line through the scatterplots from the different Erlenmeyer flasks, and calculating the formula of this trend line. For example: $y = 4.9571e^{0.1916x}$; $R^2 = 0.9721$, where 0.1916 g g^{-1} d^{-1} is the RGR value (growth of approximately 19% a day). The exponential function in Excel also calculates r^2 as a measurement of the correlation, to be statistically significant. The R^2-value describes an exponential regression over the plotted trend line. The RGR for the different nutrient concentrations are visualised in a separate graph, with the used $NaNO_3$ or $NaPO_4$ concentrations on the x-axis and the RGR on the y-axis. The highest RGR found will be used as RGRmax at 10°C in the simulation model described in 2.3.

2.3 Simulation Model

Simulation models are mathematical models used to simulate the actual field status, to consequently be able to predict future values, like the regeneration of seaweeds. Multiple *Ulva* studies have been conducted by using simulation models, especially studies about growth influenced by environmental factors (Wichard *et al*., 2015) (Lababpour, 2018). Because seaweed has an increasing market, these models become more important every day. In Dutch perspective, Wageningen University is doing research on developing and using simulation models in future seaweed development (Van 't Klooster, 2018 ; Anten, 2018). The model developed for this study is based on the simulation model from lectures by Van 't Klooster and Anten. This relatively simple model can be used for any seaweed, influenced by these factors, in any location. However, the model presented in these lectures mainly focuses on possibilities for seaweed cultivation and harvesting in the North Sea and Eastern Scheldt. The Dutch Wadden Sea is not addressed. For this reason, a new adjusted model has been constructed, custom-made for wild harvesting *Ulva spp.* in the Dutch Wadden Sea.

In the used simulation model, maximum relative growth rate (RGR) is influenced by growth influencing factors such as nitrate, phosphate and temperature. This RGR is used to calculate the increase in total biomass over a certain period of time. Table 3 until 5 describe the different calculations and parameters used in the simulation model. The maximum RGR of *Ulva* is 0.3095 g g^{-1} d^{-1}, according to Breure, 2014. This value will be used at a temperature of 15°C and 20°C, while using the reducing factor temperature, in which RGRmax will be reduced at 15°C and remain stable at 18-20°C (optimum). The actual reducing values are shown in the results (3.2.). The RGRmax following from the experiment mentioned above (2.2.) is used at a temperature of 10°C, uninfluenced by the model's reducing factor temperature.

This maximum relative growth rate is influenced (reduced) by different growth factors. The simulation model describes these conditions in present numbers from the Dutch Wadden Sea, compared with the optimum conditions of *Ulva*. The calculations in table 3 describe how to calculate these reducing factors, and consequently calculate the new RGR and biomass (B). The used reducing factors in this model are nitrate availability, phosphate availability and 3 different temperatures (10˚C, 15˚C, 20˚C), to measure seasonal differences. Nitrate and phosphate availability are chosen as stable values over time, because of the continuous influx from the Ijsselmeer and the river Eems (1.2. Area description). Besides these values, more reducing factors could be included, to increase the reliability of the model. The model in this study is based on unlimited growth, 12 hours of light per day (average) and a salinity value of 28 g/L.

The simulation model is used to calculate the regeneration of *Ulva spp.* in different environmental factors and different starting biomass stocks present in the Dutch Wadden Sea. The used wild harvest value, as seen in calculation 2 (table 3) is 1000kg per day. The calculated quantities will provide the necessary results, to be able to conclude the regeneration speed of *Ulva* in the Dutch Wadden Sea, with and without harvesting 1000kg per day. The output values of regeneration while harvesting 1000kg *Ulva* per day will be compared to output values without wild harvesting using different starting values for biomass. The starting biomass used in this study are randomly chosen numbers: 10000kg and 50000kg. These numbers are chosen randomly, because at this moment, there is no knowledge of the actual present *Ulva* biomass in the Dutch Wadden Sea, as stated in the introduction (1.2. Area description).

The consequential results will be processed into graphs, these are compared with each other and with literature. Consequently, conclusions will be formulated.

Table 3: Calculations

Equation	Formula	Description
1	RGR=RGRmax*fT*fN*fP*fS	Relative growth rate
2	B=Bt-1+Growth- 1000 (wild harvest)	Biomass in kg
3	Growth= RGR*B	Growth of seaweed biomass per day
4	FT= Tact<Top (T-Tmin)/(Top-Tmin); Tact>Top (Tmax-T)/(Tmax-Top))	Reducing factor temperature
5	FS= Sact<Sop (Sact-Smin)/(Sop-Smin); Sact>Sop (Smax-Sact)/(Smax-Sop))	Reducing factor salinity
6	FP= (Pact)/(Pact+Kp)	Reducing factor phosphate
7	FN=(Nact)/(Nact+Kn)	Reducing factor nitrogen

Table 4: Parameters

Symbol	Value	Unit	Description
RGR max	From Article: 0,3095 (Breure, M. S. 2014) From Experiments at 10°C: 0.20	g g-1 day-1 m-2	Max growth biomass per gram biomass per day
Sact	28 (Van Aken, 2008)	Gram/Litre	Actual salinity seawater
Nact	50 (Kloepper *et al.*, 2017)	µM nitrate per litre seawater	Actual nitrogen concentration seawater
Pact	1 (Kloepper *et al.*, 2017)	µM phosphate per litre seawater	Actual phosphate concentration seawater
Smin	15	Gram/Litre	Minimal salinity needed for seaweed growth
Smax	63 (Xia, J., et al. (2004).)	Gram/Litre	Maximum salinity needed for seaweed growth
Sop	28-32 (Groenendijk *et al.,* 2016)	Gram/Litre	Optimal salinity needed for seaweed growth
Tmin	5 (Groenendijk *et al.,* 2016)	Degrees °C	Minimal growth temperature
Tmax	25 (Groenendijk *et al.,* 2016)	Degrees °C	Maximal growth temperature
Top	18 (Groenendijk *et al.,* 2016)	Degrees °C	Optimal growth temperature
Kp	1.276 (Breure, 2014)	Phosphorus uptake in µM	
Kn	6 (Breure, 2014)	Nitrogen uptake in µM	

Table 5: Variables

Symbol	Unit	Description	Value
Tact	Degrees °C	Actual temperature seawater	Average of 15 in spring, average of 20 in summer (KNMI, 2019).

Table 6: Calculation quantities

Symbol	Unit	
RGR	g g-1 day-1 m-2	Growth biomass per gram biomass per day
Bt	Kg (dm) /ha	Total biomass at a certain day (t)

2.4 Literature study

This study is partly based on literature study and partly on own results. Only a part of the models input is from the conducted lab experiments. The other part is from literature study. This is done because most input values can't be extracted from the experiment.

Literature has been collected using multiple tools, namely WUR Library, Google Scholar, present books in the WMR laboratory/library and expertise from employees of WMR, especially Jak, 2019. Literature study will be conducted using a literature search plan, described in Appendix II. Most of the used literature was found using the keywords (table 7) from this plan, some articles were pointed out by seaweed expert and supervisor Robbert Jak. These keywords have been used, because all of them are in line with the studied subject. The most information was extracted from the QSR (Quality Status Report) Wadden Sea. (Thieltges, 2017).

Table 7: Keywords used in searching literature

Ulva (and all species, for example *Ulva rigida*)	Seaweed
RGR (Relative growth rate)	Use of seaweed in different countries
Salinity	Seaweed cultivation
Temperature	Wild harvest
Light intensity	European seaweed
Nutrients	Dutch seaweed
Simulation models	Quality status report Dutch Wadden Sea
F-2 Medium	Sea Agriculture
Animal feed	Human consumption of seaweed
Growing conditions *Ulva*	Macroalgae

3. Results

<u>What is the maximum relative growth rate (RGR) of Ulva grown in which nitrate and phosphate concentrations?</u>

3.1. RGRmax experiments with nitrate and phosphate

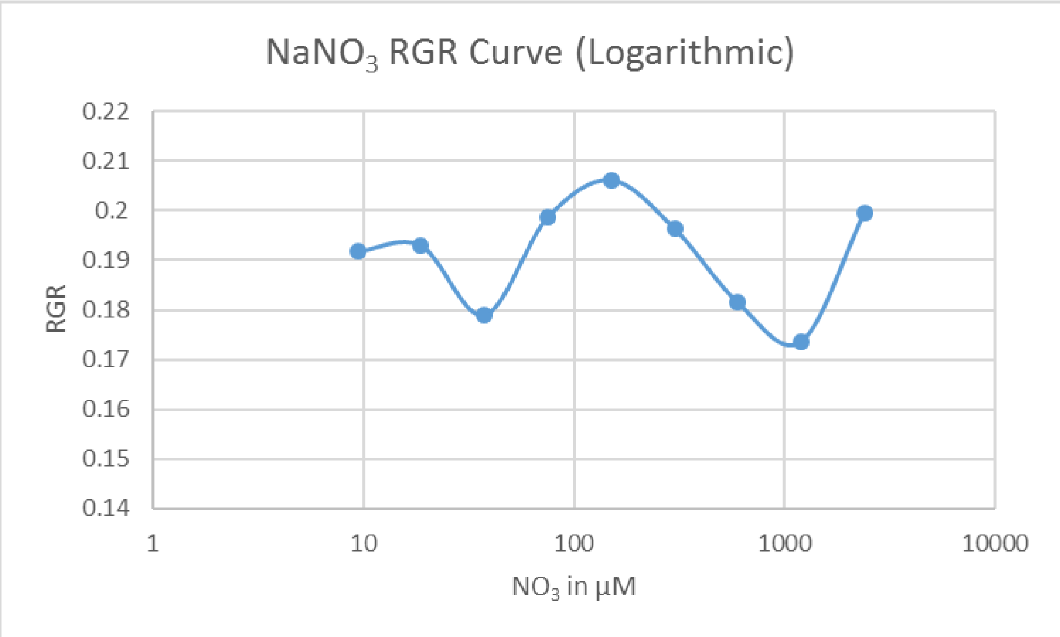

Figure 7: $NaNO_3$ RGR Curve (Logarithmic)

Figure 7 shows the RGR of *Ulva* in different nitrate concentrations. As seen in this figure, the RGR differs quite a lot between different concentrations. The highest RGR is measured using a concentration of 150 µM/L $NaNO_3$, which is 0.2062 g g^{-1} d^{-1}. The graph shows an optimum curve around this optimum nitrate concentration, with an outlier on the highest concentration: 2400 µM/L $NaNO_3$. Appendix III shows the growth curves per Erlenmeyer flask, so per $NaNO_3$ concentration. Figure 7 is based on the formulas of the plotted trend lines shown in Appendix III. Appendix III also shows the goodness of fit of the trend line, amongst the different *Ulva* disks per Erlenmeyer.

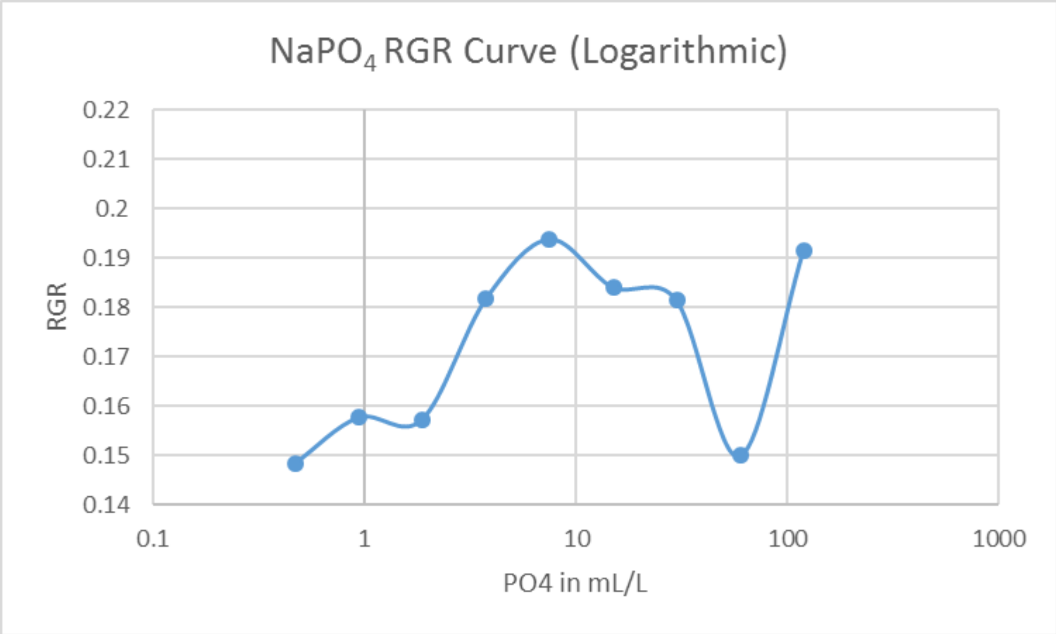

Figure 8: NaPO₄ RGR Curve (Logarithmic)

Figure 8 shows the RGR of *Ulva* in different phosphate concentrations. As seen in this figure, the RGR differs quite a lot between different concentrations. The highest RGR is measured using a concentration of 7.5 µM/L $NaPO_4$, which is 0.1939 g g^{-1} d^{-1}. The graph shows an optimum curve around this optimum phosphate concentration, with an outlier on the highest concentration: 120 µM/L $NaPO_4$. Appendix IV shows the growing curves per Erlenmeyer flask, so per $NaPO_4$ concentration. Figure 8 is based on the formulas of the plotted trend lines shown in Appendix IV.

3.2. Influence of reducing factors on RGRmax

Which reducing factors have the most influence on the relative growth rate (RGR) of *Ulva* in the Dutch Wadden Sea?

Table 8: Ulva RGR influenced by reducing factors in the simulation model

	RGR	***fT***	***fN***	***fP***
Uninfluenced by reducing factors	0.310	1 (T=18-20)	1	1
Influenced by reducing factors	0.121	1 (T=18-20)	0.89	0.44
	0.093	0.77 (T=15)	0.89	0.44
	0.079	(T=10) (Experiment RGR used)	0.89	0.44

Table 8 shows RGR influenced and uninfluenced by reducing factors. During spring, summer the reducing factor fT (temperature) fluctuates during the simulated time, because the water temperature differs in this time period (T=10, T=15, T=20).

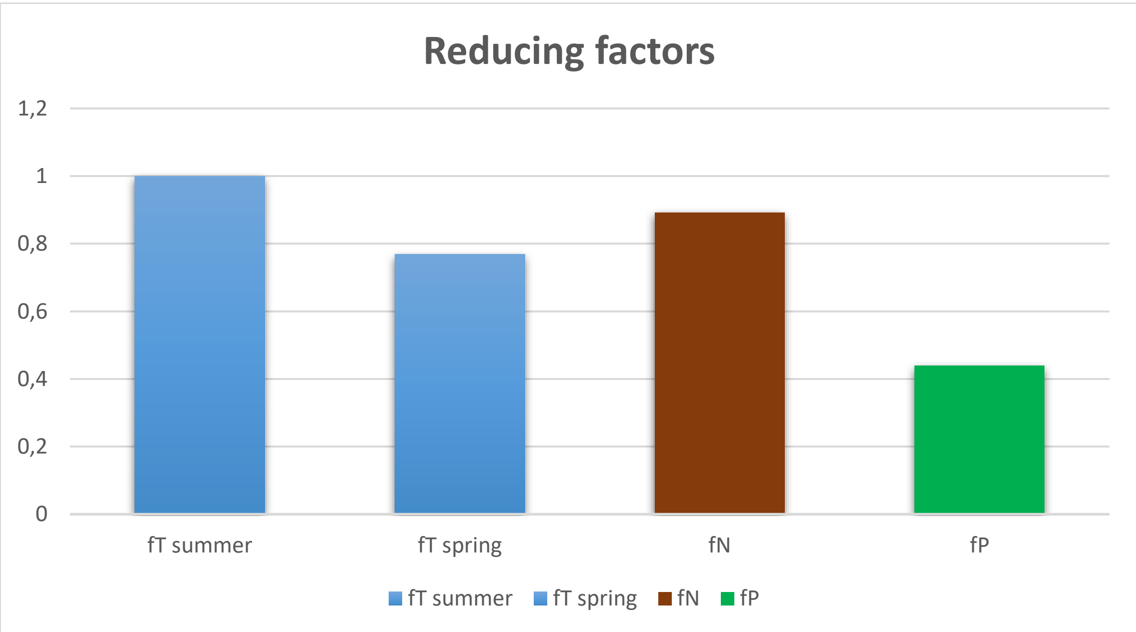

Figure 9: Reducing factor values

Figure 9 shows the reducing factors on RGR. The lower the reducing factor, the higher its influence on the RGR. The greatest reducing factor in this study is fP. fT=10 is not included in this graph, because the value is derived from experiments.

3.3. Output values of simulation model in various situations

<u>What is the difference in *Ulva* regrowth after 1000kg wild harvest, comparing to a situation where there is no wild harvest, according to the used simulation model?</u>

<u>What is the difference in biomass output, using different seasonal temperatures (10˚C, 15˚C, 20˚C)?</u>

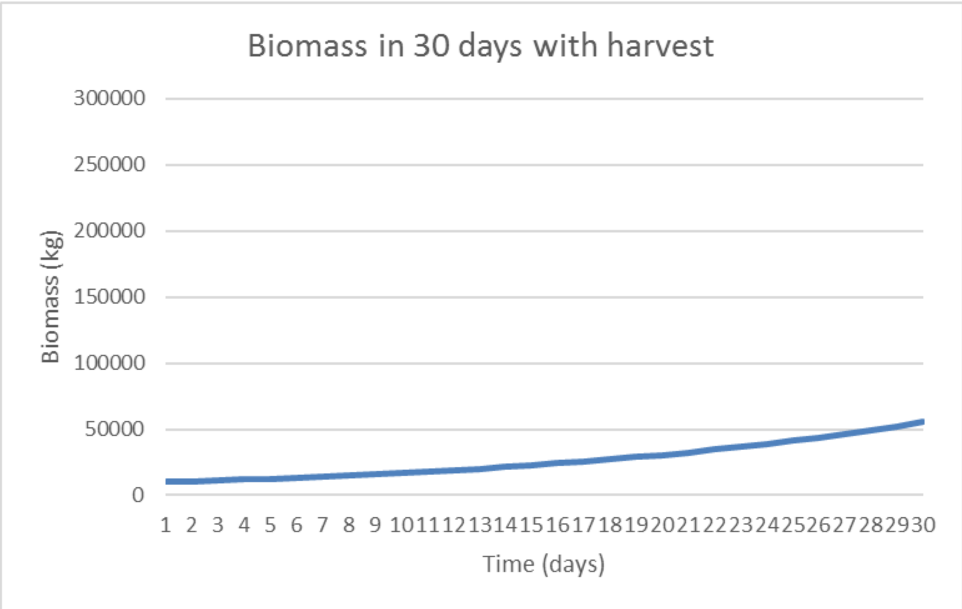

Figure 10: Increase in biomass in 30 days while wild harvesting 1000kg per day (based on unlimited growth). In this graph T=18-20, so the RGR is 0.12. Starting biomass of the Dutch Wadden Sea B=10000kg.

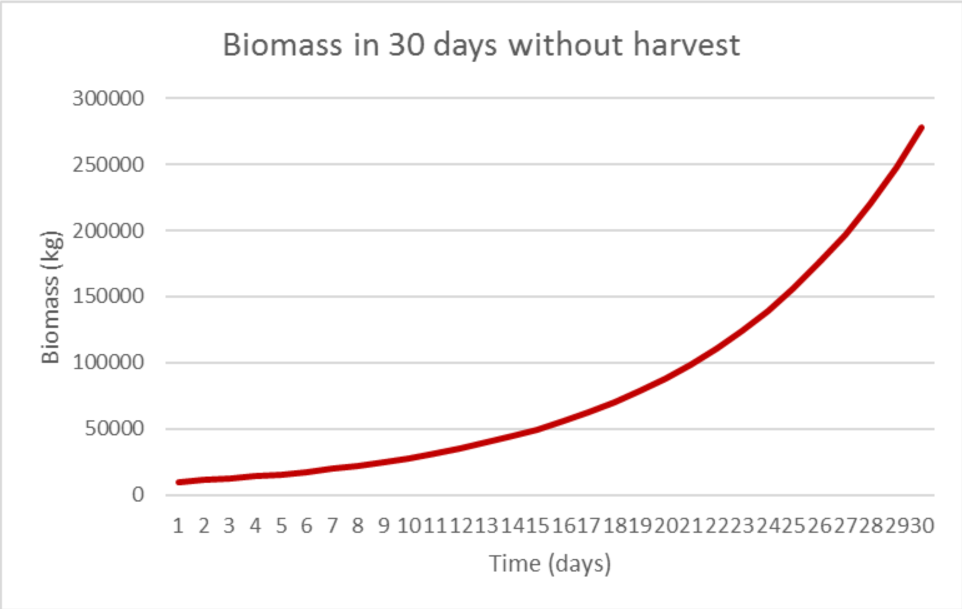

Figure 11: Increase in biomass in 30 days without wild harvesting 1000kg per day (based on unlimited growth). In this graph T=18-20, so the RGR is 0.12. Starting biomass of the Dutch Wadden Sea B=10000kg.

Figures 10 and 11 show the increase in biomass, based on unlimited growth, in a time scale of one month, with a starting biomass of 10000 (B0=10000). With a daily harvest of 1000kg, the biomass increases from 10000kg till almost 60000kg *Ulva* in the Dutch Wadden Sea. When there is no wild harvest, the total biomass increases from 10000kg till approximately 275000kg. The increase in biomass is almost 5 times as big as the increase with a daily harvest of 1000kg.

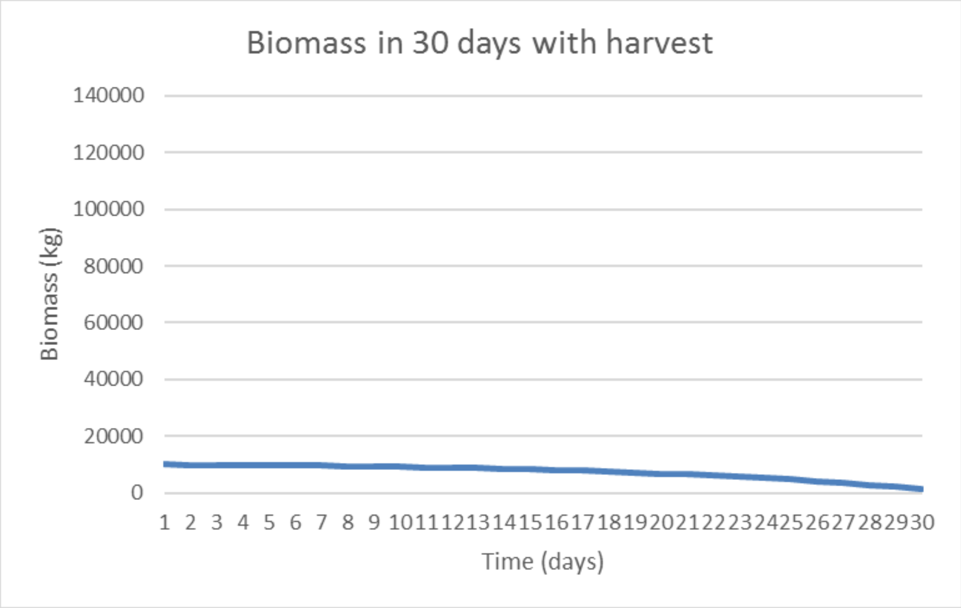

Figure 12: Increase in biomass in 30 days while wild harvesting 1000kg per day (based on unlimited growth). In this graph T=15, so the RGR is 0.093.). Starting biomass of the Dutch Wadden Sea B=10000kg.

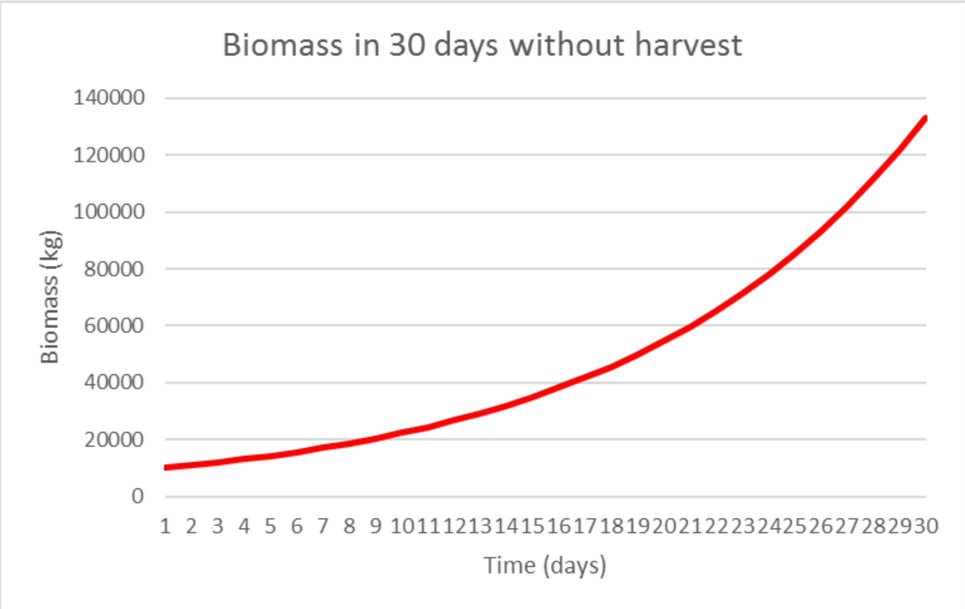

Figure 13: Increase in biomass in 30 days without wild harvesting 1000kg per day (based on unlimited growth). In this graph T=15, so the RGR is 0.093. Starting biomass of the Dutch Wadden Sea B=10000kg.

Figures 12 and 13 show the increase in biomass, based on unlimited growth, in a time scale of one month, with a starting biomass of 10000 (B0=10000). In this scenario (T=15), the biomass decreases due to wild harvest. The total biomass will almost reach 0 after 1 month of harvesting (figure 12). When there is no wild harvest, the total biomass increases from 10000kg till approximately 130000kg.

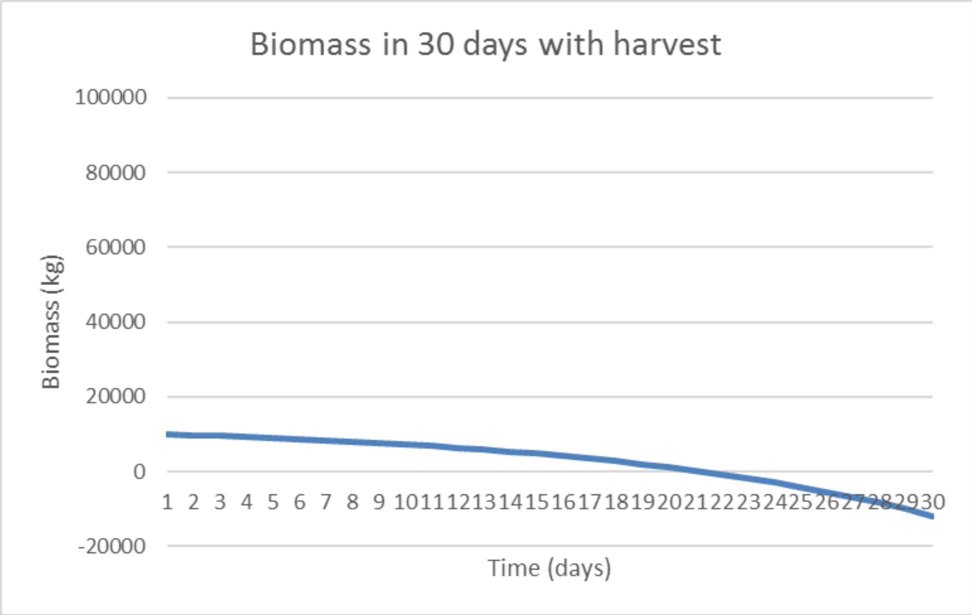

Figure 14: Increase in biomass in 30 days while wild harvesting 1000kg per day (based on unlimited growth). In this graph T=10, so the RGR is 0.078. Starting biomass of the Dutch Wadden Sea B=10000kg.

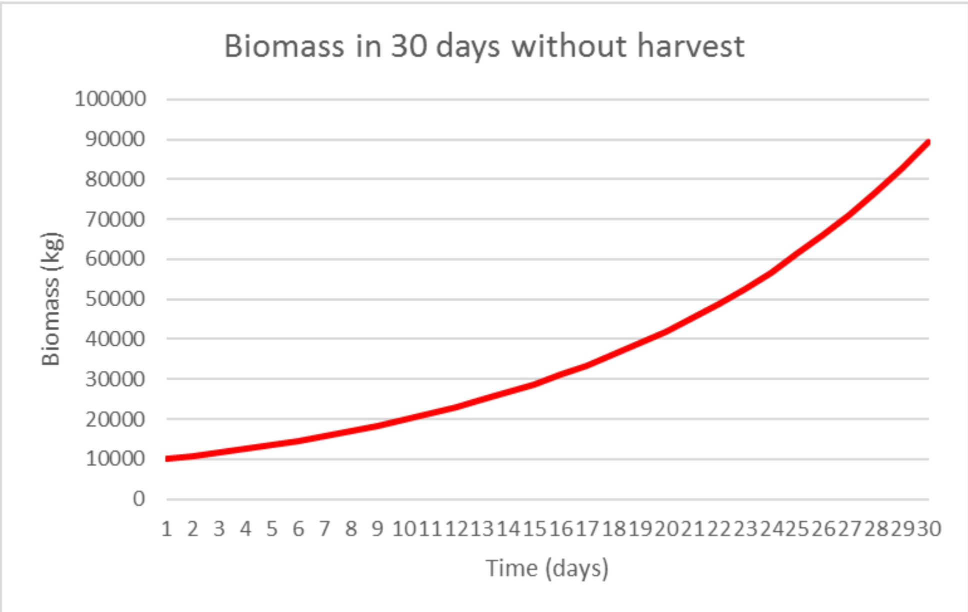

Figure 15: Increase in biomass in 30 days while wild harvesting 1000kg per day (based on unlimited growth). In this graph T=10, so the RGR is 0.078. Starting biomass of the Dutch Wadden Sea B=10000kg.

Figures 14 and 15 show the increase in biomass, based on unlimited growth, in a time scale of one month, with a starting biomass of 10000 (B0=10000). In this scenario (T=10), the biomass decreases due to wild harvest. The total biomass will reach below 0 after 23 days of harvesting (figure 14). When there is no wild harvest, the total biomass increases from 10000kg till approximately 90000kg.

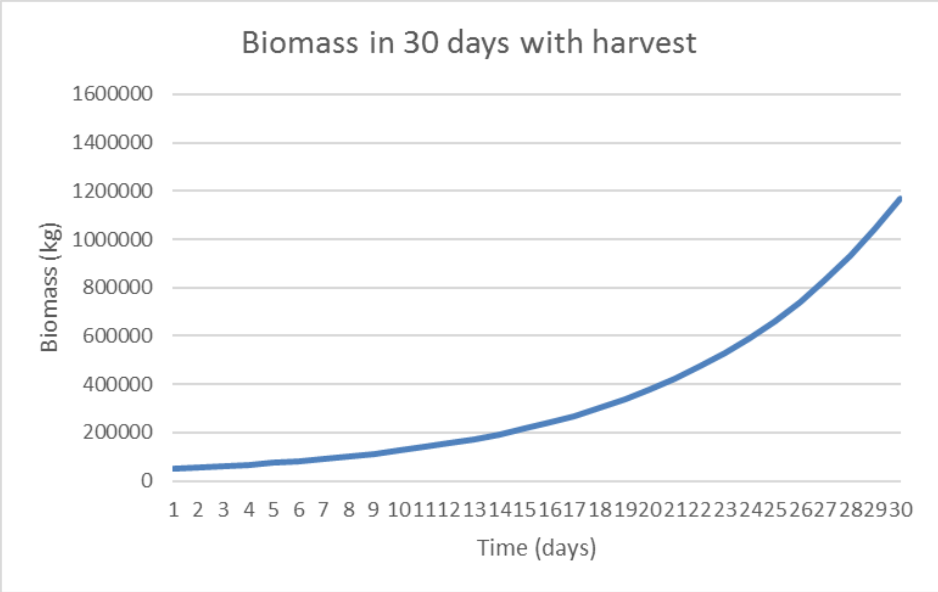

Figure 16: Increase in biomass in 30 days while wild harvesting 1000kg per day (based on unlimited growth). In this graph T=18-20, so the RGR is 0.12. Starting biomass of the Dutch Wadden Sea B=50000kg.

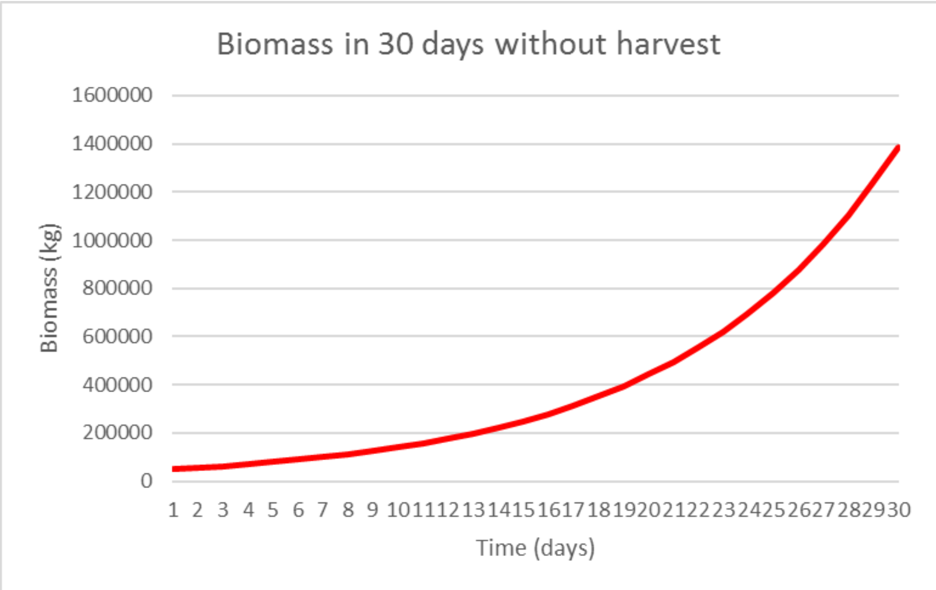

Figure 17: Increase in biomass in 30 days without wild harvesting 1000kg per day (based on unlimited growth). In this graph T=18-20, so the RGR is 0.12. Starting biomass of the Dutch Wadden Sea B=50000kg.

Figures 16 and 17 show the increase in biomass, based on unlimited growth, in a time scale of one month, with a starting biomass of 50000 (B0=50000). In this scenario (T=20), with a daily harvest of 1000kg, the biomass increases from 50000 kg till approximately 1.2 million kg *Ulva* in the Dutch Wadden Sea. When there is no wild harvest, the total biomass increases from 50.000kg till approximately 1.4 million kg. The increase in biomass is only 1.2 times as big as the increase with a daily harvest of 1000kg.

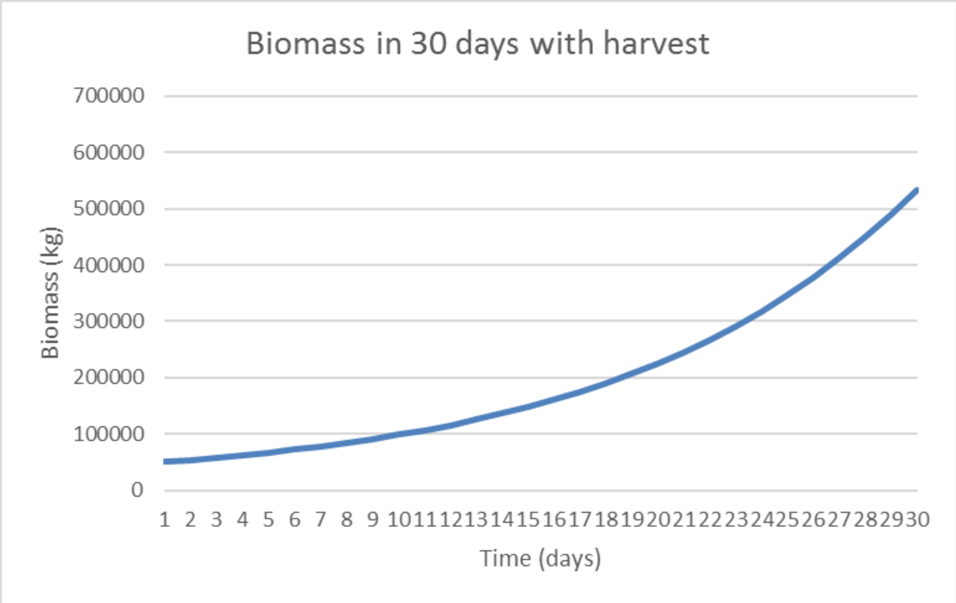

Figure 18: Increase in biomass in 30 days while wild harvesting 1000kg per day (based on unlimited growth). In this graph T=15, so the RGR is 0.093. Starting biomass of the Dutch Wadden Sea B=50000kg.

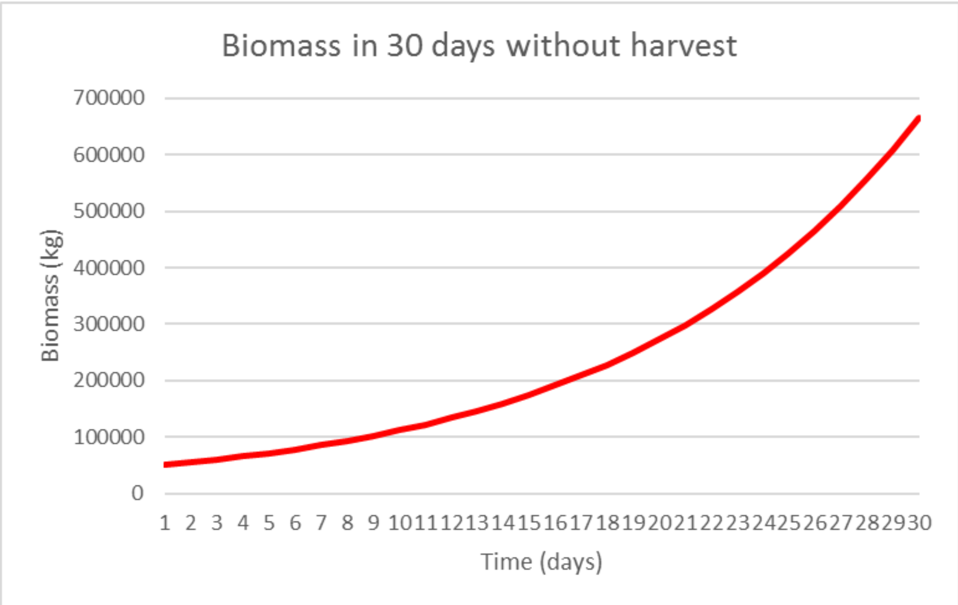

Figure 19: Increase in biomass in 30 days without wild harvesting 1000kg per day (based on unlimited growth). In this graph T=15, so the RGR is 0.093. Starting biomass of the Dutch Wadden Sea B=50000kg.

Figures 18 and 19 show the increase in biomass, based on unlimited growth, in a time scale of one month, with a starting biomass of 50000 (B0=50000). In this scenario (T=15), with a daily harvest of 1000kg, the biomass increases from 50000 kg till approximately 550000 kg *Ulva* in the Dutch Wadden Sea. When there is no wild harvest, the total biomass increases from 50000kg till approximately 670000 kg. The increase in biomass is 1.22 times as big as the increase with a daily harvest of 1000kg.

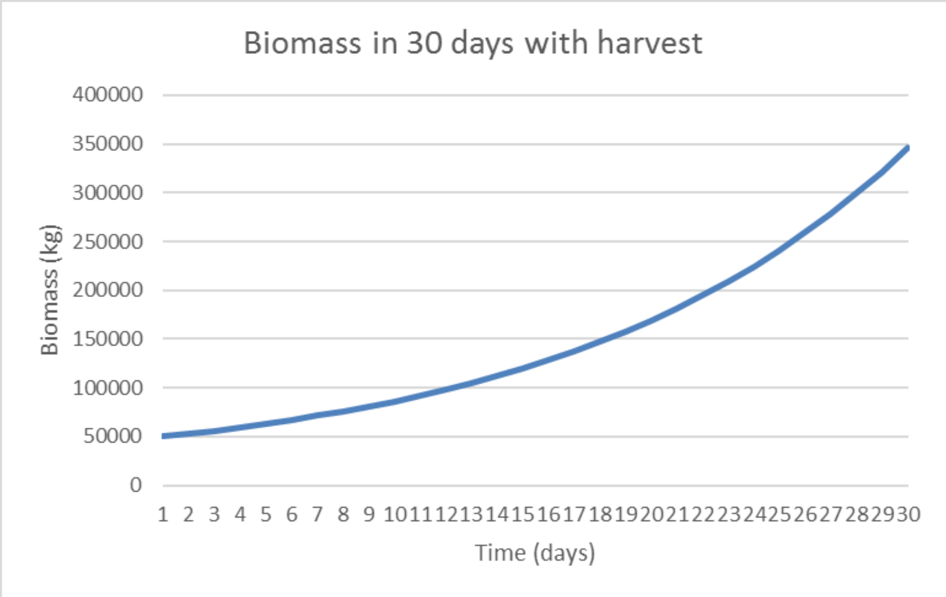

Figure 20: Increase in biomass in 30 days while wild harvesting 1000kg per day (based on unlimited growth). In this graph T=10, so the RGR is 0.078. Starting biomass of the Dutch Wadden Sea B=50000kg.

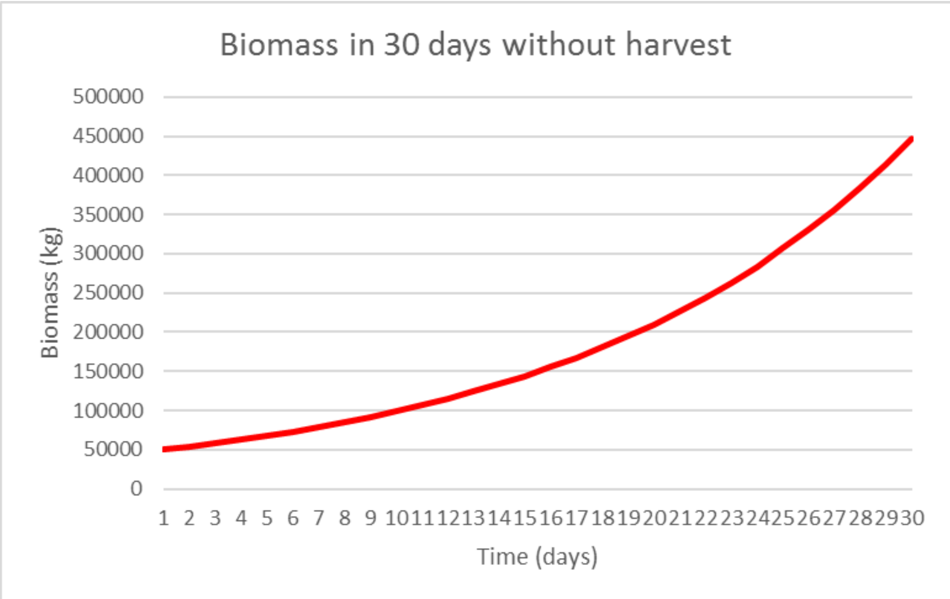

Figure 21: Increase in biomass in 30 days while wild harvesting 1000kg per day (based on unlimited growth). In this graph T=10, so the RGR is 0.078. Starting biomass of the Dutch Wadden Sea B=50000kg.

Figures 20 and 21 show the increase in biomass, based on unlimited growth, in a time scale of one month, with a starting biomass of 50000 (B0=50000). In this scenario (T=10), with a daily harvest of 1000kg, the biomass increases from 50000 kg till approximately 350000 kg *Ulva* in the Dutch Wadden Sea. When there is no wild harvest, the total biomass increases from 50000kg till approximately 450000 kg. The increase in biomass is 1.29 times as big as the increase with a daily harvest of 1000kg.

4. Discussion

4.1. Nitrate/phosphate experiments

The outcomes of the experiments with different nitrate and phosphate concentrations show the highest RGR by using the optimum concentrations described by Gao, 2016, as expected. However, the graph does not show a proper optimum curve, mostly due to the high RGR values with extremely high nitrate and phosphate concentrations. This unexpected high RGR could have happened due to multiple possible reasons:

- Flaws in mixing the F-2 medium: there could've been mistakes in pipetting the proper concentrations into the flasks, or the concentrations could've been influenced by contamination.
- Coincidence: The *Ulva* disks added to the highest concentrations could have been absorbing more light, or be fresher, faster growing parts of the *Ulva* leaf, although disks have been randomly distributed from multiple *Ulva* leaves.

The different RGR values are measured using a plotted trend line through the different *Ulva* disks at different moments in time. The R^2-value of these trend lines was mostly pretty high, what indicates that the goodness of fit between different disks is quite high. This indicates a statistical significance amongst these different disks. This R^2-value might have been different if more disks survived, because now in some cases, only 1 or 2 disks survived, which automatically increases the goodness of fit, due to lack of comparing material. The amount of surviving disks is shown in Appendices III and IV. The amount of plotted points indicates the amount of surviving disks per time unit. These Appendices also show the R^2-value per Erlenmeyer flask.

The RGR values found on the optimums were not as high as RGRmax found by Breure, 2014. This is probably because the growing conditions, besides nitrate and phosphate, were not optimal. Firstly, the temperature was set on 10°C instead of 18-20°C, to leave out the possibility of temperature shock. This temperature shock happened during a try-out experiment at 18°C, all the disks emptied their cells and died. Secondly, the light intensity in the climate chamber was lower than the optimum: 6000 Lux (Gao, 2016). This value was measured around 5000 Lux. The salinity might have had influence as well, as the used filtered seawater originated from the Eastern Scheldt, although this seems unlikely, because the salinity of the Eastern Scheldt lies around 32-33 g/L (De Vries, 2015). This is why the RGRmax of 0.3095 g g^{-1} d^{-1} from Breure, 2014 was used at temperatures 15°C (spring) and 18-20°C (summer). The experiments' RGRmax is used at a temperature of 10°C.

During the experiment, another method of measuring *Ulva* growth was tested. In this method, the growth was not measured by weight, but by surface. The surface method turned out to be slower and even more insecure than measurement by weight.

4.2. Simulation Model

In this study, a couple of possible input values have been used in the simulation model. The model is based on unlimited *Ulva* growth, influenced by some reducing factors.

At this moment in time, the simulation model is not reliable enough to truthfully estimate the actual field status. Although the simulation model could represent *Ulva* growth in certain circumstances, there are still many extra options to include, such as more reducing factors. An example of other influential factors could be: light availability, mortality, turbidity, wave intensity, presence of shipping, predation, competition etcetera. Besides this, the model is based on unlimited *Ulva* growth, while the Dutch Wadden Sea might not have the capacity for a continuously increasing amount of *Ulva* biomass.

- In the case of light availability, the amount of hours of light per day fluctuate over time and thus differ per season. This could be implemented in the model as another reducing factor, with a reduction of RGRmax when light availability is not optimal (optimum *Ulva:* 16 hours of light per day) and light intensity is not optimal (80 µmol photons m^{-2} s^{-1}). (Gao, 2016).
- Mortality could be included in the model as a variable, reducing the present biomass per day by a certain percentage. The value of mortality could also be linked to other influential factors, like the RGRmax is being influenced in the current model.
- Turbidity could be included as solid reducing factor, like nitrate and phosphate in this study's model.
- Wave intensity and presence of shipping are pretty hard to implement in the model in actual numbers, because the influence of these factors is influenced itself, for example by factors as wind, flow rate and coincidence.
- Predation and competition could be included as solid numbers, reducing the biomass per day by a certain percentage. However, these factors are harder to measure than light availability and mortality.

These factors could also influence each other, which makes it even harder to implement as many factors as possible in the simulation model. For example, wave intensity could influence turbidity and mortality. This could also be included in a future version of the used model.

The most important fluctuating reducing value in the model is the water temperature. This value could influence the RGR a lot due to seasonal changes. The best moment to wild harvest *Ulva* would be with an optimal water temperature of 20°C, because then the reducing factor temperature has the least impact. However, this is purely based on this study's simulation model, which doesn't represent all possible influential factors.

The nitrate and phosphate values could fluctuate as well, but this fluctuation would be based on other influential factors, which are not included in this study. For this reason constant nitrate and phosphate values have been used in the model. Winter values of both nutrients and water temperature are not included, because *Ulva* doesn't grow in the Dutch Wadden Sea in this season, due to low water temperatures and light intensity (Groenendijk *et al.,* 2016).

The most important missing value in this study is the total *Ulva* biomass in kilograms in the Dutch Wadden Sea at a certain moment of time. The biomass input values used (10000kg and 50000kg) are randomly picked (although presence is based on observations) and might not be representing actual present values. However, based on using these input values, the output values lead to a solid conclusion, which has little to do with the actual present biomass in the Dutch Wadden Sea. This conclusion states that with an increasing amount of starting biomass, wild harvesting 1000 kg per day will have a lower impact on the population. So, before the actual wild harvesting can start, the total *Ulva* biomass in the Dutch Wadden Sea has to be calculated, which can be done in further research.

Adding to that, in the near future, Wageningen Marine Research is planning to conduct studies to the actual present *Ulva* biomass in the Dutch Wadden Sea. This means the simulation model could be used for the actual field values. This means that, in the near future, the simulation model in this study, with added reducing values as described above, could be used for reliable estimations in the Dutch Wadden Sea. After that, the wild harvesting of *Ulva* from the Wadden Sea could actually start.

The amount that will be hypothetically wild harvested in this study (1000kg), is, as mentioned in the introduction (1.1.), not based on facts, but merely on random picking. For now, actual wild harvesting values are not possible, because of lack of the starting biomass present in the Dutch Wadden Sea. The European Natura 2000 law system could also be a problem in the actual amounts of wild harvest, because the chosen area is protected. For actual wild harvest to happen, consultation with the Dutch and maybe European government has to be held. After these discussions, agreements about the wild harvest per day could be set up.

Although *Ulva* might be the main subject of this study, the model could be used with any species of seaweed. As long as enough proper input values are used, any measurement of seaweed growth is possible.

5. Conclusion

The main purpose of this study was to simulate *Ulva* growth in certain circumstances in the Dutch Wadden Sea, using a therefore created model, to be able to see the influence of wild harvesting *Ulva* biomass from the Dutch Wadden Sea. This lead to the main question:
What is the impact of wild harvesting *Ulva spp.* biomass on its population in the Dutch Wadden Sea?
This question was supported by sub-questions:

What is the maximum relative growth rate (RGR) of *Ulva* grown in which nitrate and phosphate concentrations?
The RGR of *Ulva* in the Dutch Wadden Sea was measured the highest at the optimum concentrations of both nitrate (150 µM/L $NaNO_3$) and phosphate (7.5 µM/L $NaPO_4$) This both lead to a maximum RGR value at 10°C of approximately 0.20 g g^{-1} d^{-1}.

What is the difference in *Ulva* regrowth after 1000kg wild harvest, comparing to a situation where there is no wild harvest, according to the used simulation model?
The conclusions of the model are that with a higher amount of present biomass, the influence of wild harvesting is a lot less destructive for the population. The restoration of the population could differ with a factor 5 when the harvesting is done with a low amount of present biomass. With a starting biomass of 10000 kg, the impact of wild harvesting 1000kg per day for one month would lead to a reduction of the *Ulva* population by a factor 5, while starting with a biomass of 50000kg, the population will only decrease by a factor 1.2 after one month of wild harvesting 1000kg per day.

What is the difference in biomass output, using different seasonal temperatures (10˚C, 15˚C, 20˚C)?
Wild harvesting has more effect with a lower water temperature, due to slower regeneration of the population. At a starting biomass of 10000kg, wild harvesting 1000kg per day with a temperature of 15°C or 10°C leads to a decrease in the *Ulva* biomass, eventually leading to stock depletion in the Dutch Wadden Sea. At a starting biomass of 50000, lower water temperatures lead to a bigger impact of wild harvesting on the regeneration of *Ulva* populations. At 20°C, the regeneration is a factor 1.2 slower, at 15°C, the regeneration is slower by a factor 1.22 and at 10°C, the regeneration is slower by a factor 1.29.

Which reducing factors have the most influence on the relative growth rate (RGR) of *Ulva* in the Dutch Wadden Sea?
This model's most influencing reducing factors on the relative growth rate of *Ulva* in the Dutch Wadden Sea were phosphate as a solid reducing factor and water temperature as a fluctuating reducing factor. In the calculations used for this study, fP (phosphate reducing

factor) turned out to be 0.44 and fT (Temperature reducing factor) turned out to fluctuate during the seasons, with a value of approximately 0.77 in spring and 1 in summer.

Main question: When has wild harvesting *Ulva spp.* biomass the least impact on its population in the Dutch Wadden Sea?

The main conclusion of the study is that with a higher amount of present biomass and with a temperature closer to the optimum, wild harvesting *Ulva* has less impact on the population. This leads to the following recommendation: for wild harvesting to be environmentally viable, the total biomass in the Dutch Wadden Sea should be high enough, to minimize negative impact. In addition, wild harvesting could be done in summer to ensure a higher RGR.

Further recommendations

To be able to predict the actual biomass values in the Dutch Wadden Sea, further research is needed. Besides that, in further studies, more reducing factors could/should be included in the simulation model, to increase the reliability. The amount of *Ulva* that will be wild harvested could also be linked to the actual amounts of human/animal consumption.

Further research could be conducted to the long term environmental impact of wild harvesting seaweeds from both the Wadden and the North Sea, using changing abiotic and biotic reducing factors over a long period of time.

Another possible future study is to use the model with North Sea values, to compare these results with the Wadden Sea and subsequently decide in which sea wild harvesting *Ulva* would be more economically and environmentally viable.

At last, further research in developments of seaweed cultivation and harvesting is always a great possibility, due to its growing demand and possibilities, on both economic and environmental fields.

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Appendices

Appendix I: Normal F-2 Medium values (Guillard & Ryther, 1962 ; Guillard, 1975)

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
$NaNO_3$	75 g/L dH_2O	1 mL	8.82×10^{-4} M
$NaH_2PO_4\ H_2O$	5 g/L dH_2O	1 mL	3.62×10^{-5} M
$Na_2SiO_3\ 9H_2O$	30 g/L dH_2O	1 mL	1.06×10^{-4} M
trace metal solution	(see recipe below)	1 mL	---
vitamin solution	(see recipe below)	0.5 mL	---

Figure 5: F-2 Medium substances

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
$FeCl_3\ 6H_2O$	---	3.15 g	1.17×10^{-5} M
$Na_2EDTA\ 2H_2O$	---	4.36 g	1.17×10^{-5} M
$CuSO_4\ 5H_2O$	9.8 g/L dH_2O	1 mL	3.93×10^{-8} M
$Na_2MoO_4\ 2H_2O$	6.3 g/L dH_2O	1 mL	2.60×10^{-8} M
ZnSO4 $7H_2O$	22.0 g/L dH_2O	1 mL	7.65×10^{-8} M
$CoCl_2\ 6H_2O$	10.0 g/L dH_2O	1 mL	4.20×10^{-8} M
$MnCl_2\ 4H_2O$	180.0 g/L dH_2O	1 mL	9.10×10^{-7} M

Figure 6: Trace metal solution substances

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
thiamine HCl (vit. B_1)	---	200 mg	2.96×10^{-7} M
biotin (vit. H)	1.0 g/L dH_2O	1 mL	2.05×10^{-9} M
cyanocobalamin (vit. B_{12})	1.0 g/L dH_2O	1 mL	3.69×10^{-10} M

Figure 7: Vitamins in F-2 Medium

Appendix II: Searchplan

Searchplan Literature study

Step 1: Het formuleren van de zoekvraag

Determine the main question. Divide the main question in subquestions which contain an aspect of the subject.

Main question:

- ➢ **What is the impact of wild harvesting *Ulva spp.* biomass on its population in the Dutch Wadden Sea?**

- **Subquestions**
  - ➢ **What is the relative growth rate (RGR) of Ulva grown in different nitrate and phosphate concentrations?**
  - ➢ **How much time does Ulva need to regrow after a certain amount of wild harvest, according to the used simulation model for the Dutch Wadden Sea? (e.g. harvesting 1000kg per day)**
  - ➢ **Which reducing factors have the most influence on the relative growth rate (RGR) of Ulva in the Dutch Wadden Sea?**

Step 2: Global delimitation of the subject

Period: February 2019 – June 2019

Location: Wageningen Marine Research, Den Helder

Language: English

Step 3: Subject orientation

Study the subject using: reference work, books, readers, articles including their bibliographies etc. Note keywords, author names, titels of key publications etc.

Keywords	Author names	Keypublications	Organisations
Ulva	Gao, 2016	Gao, 2016	NIOZ
RGR (Relative growth rate)	Robbert Jak	Jak, 2019	WMR
Salinity	Niels Anten		Waddenvereniging
Temperature	Van 't Klooster		WUR
Light intensity	Guillard & Ryther		
Nutrients			
Simulation models			
F-2 Medium			

Etc.			

Step 4: Draw up definitive list of Keywords

Ulva (and all species, for example *Ulva rigida*)	Seaweed
RGR (Relative growth rate)	Use of seaweed in different countries
Salinity	Seaweed cultivation
Temperature	Wild harvest
Light intensity	European seaweed
Nutrients	Dutch seaweed
Simulation models	Quality status report Dutch Wadden Sea
F-2 Medium	Sea Agriculture
Animal feed	Human consumption of seaweed
Growing conditions *Ulva*	Macroalgae

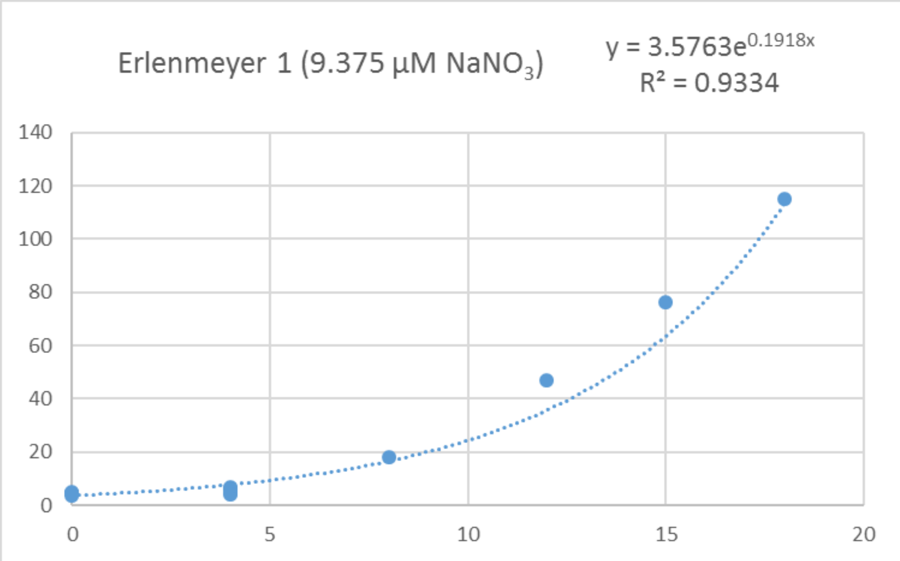
Step 5: Applied Articles

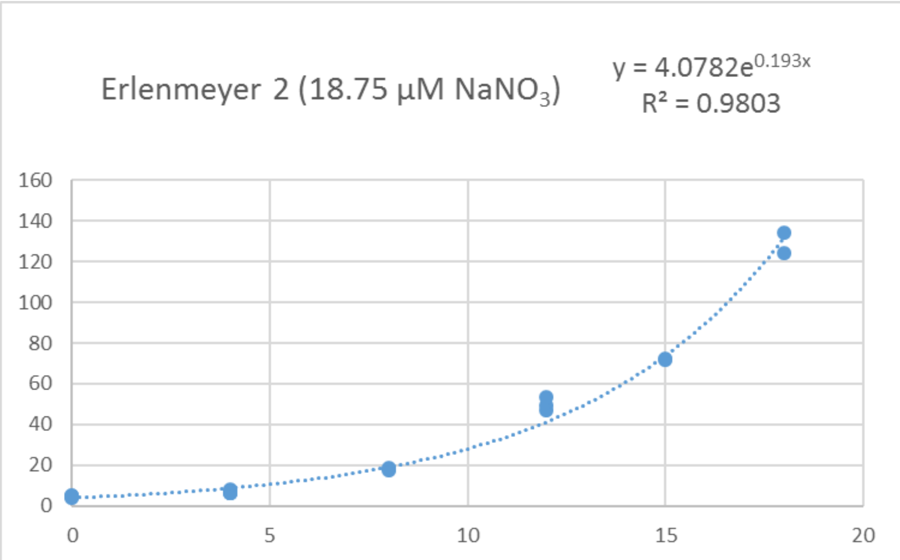
(See Bibliography)

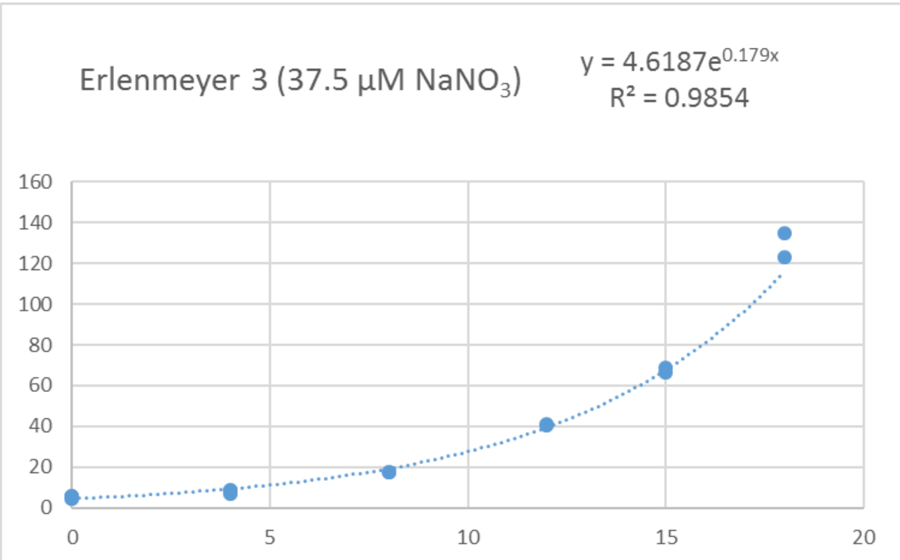
Collected from:

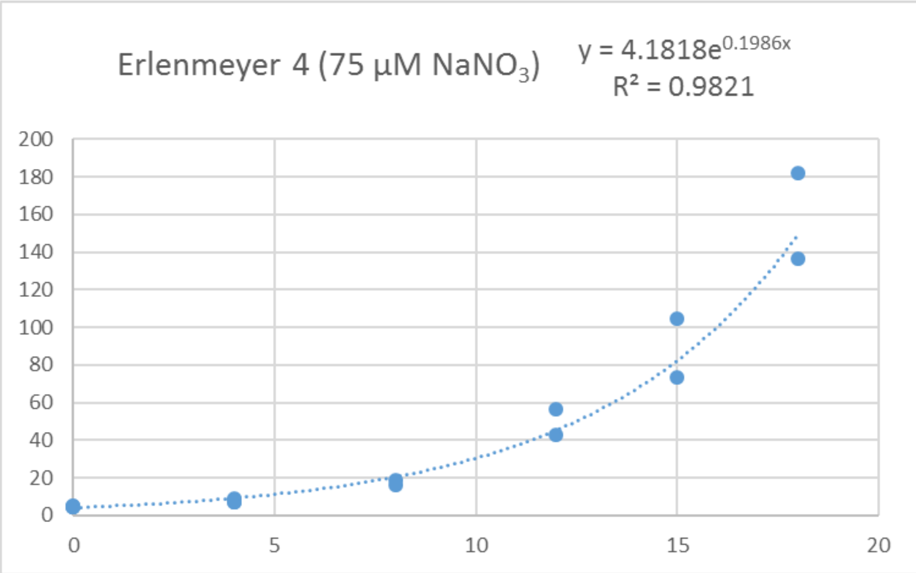
- WUR Library (using keywords from step 4)
- Google Scholar (using keywords from step 4)
- Present books in the WMR laboratory/library
- Expertise from employees of WMR, especially Jak, 2019.

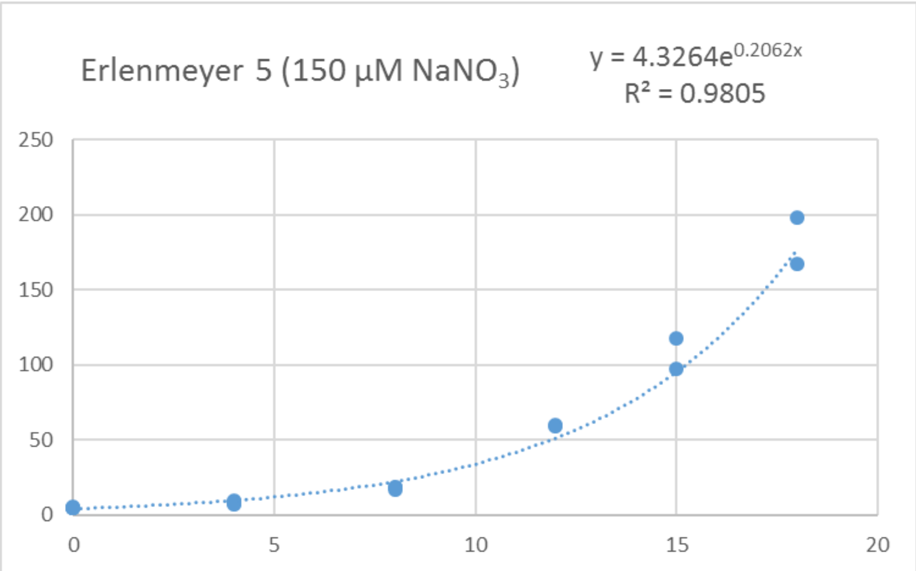
Appendix III: RGR Curves per Erlenmeyer Flask ($NaNO_3$)

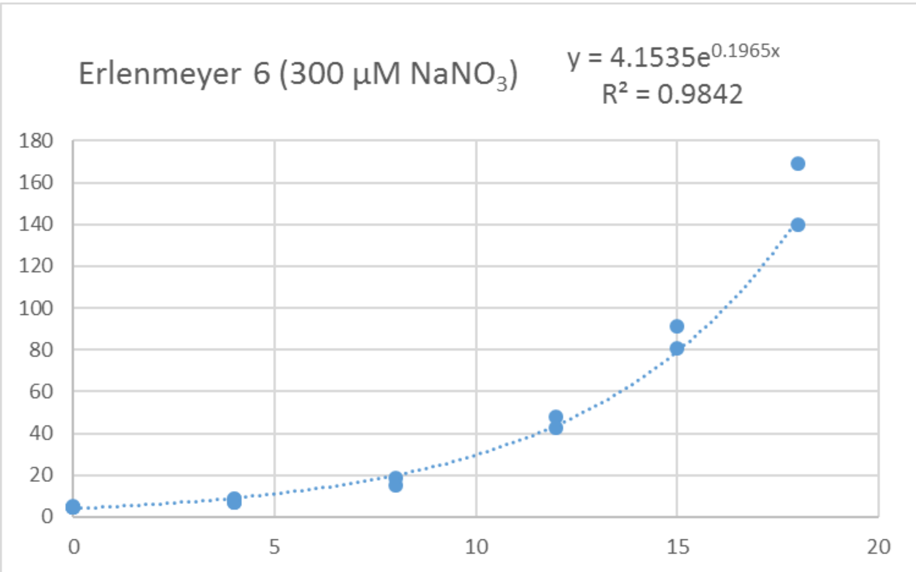


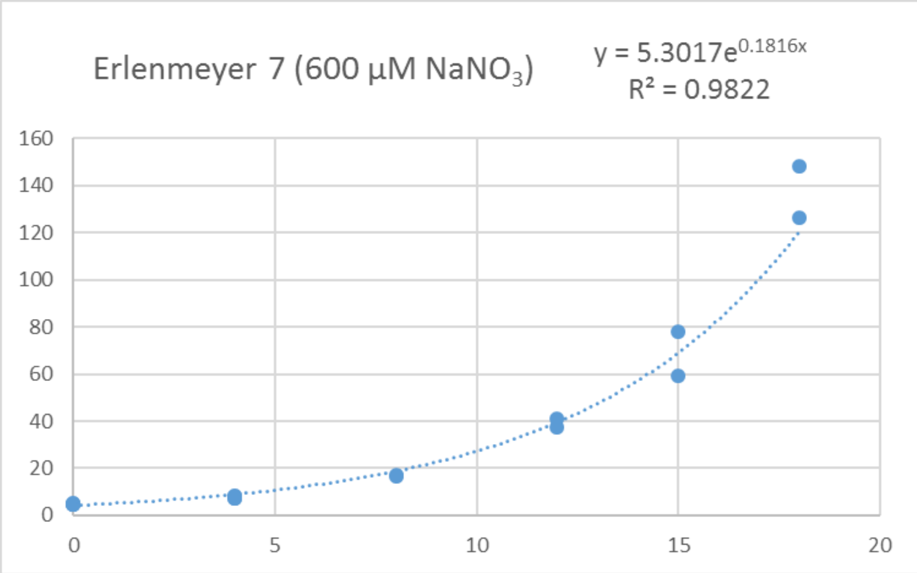


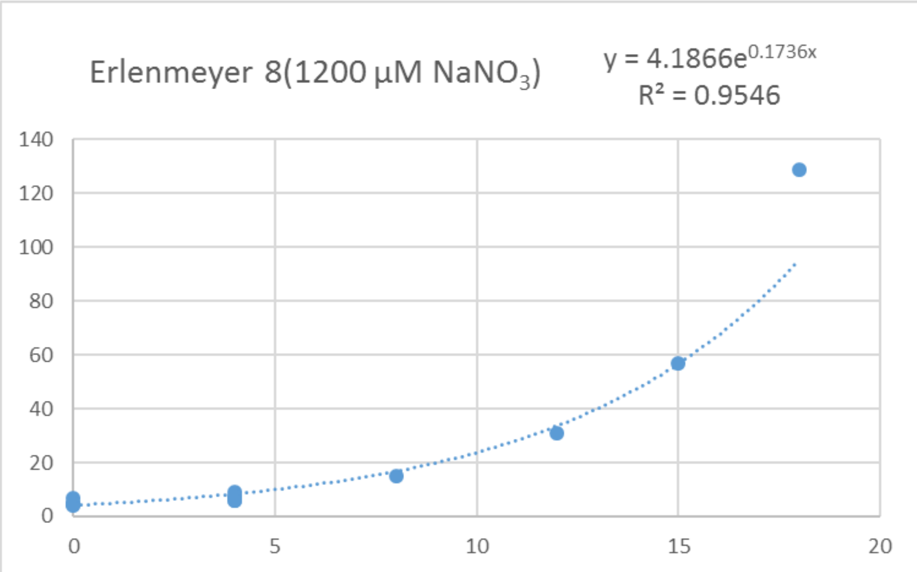


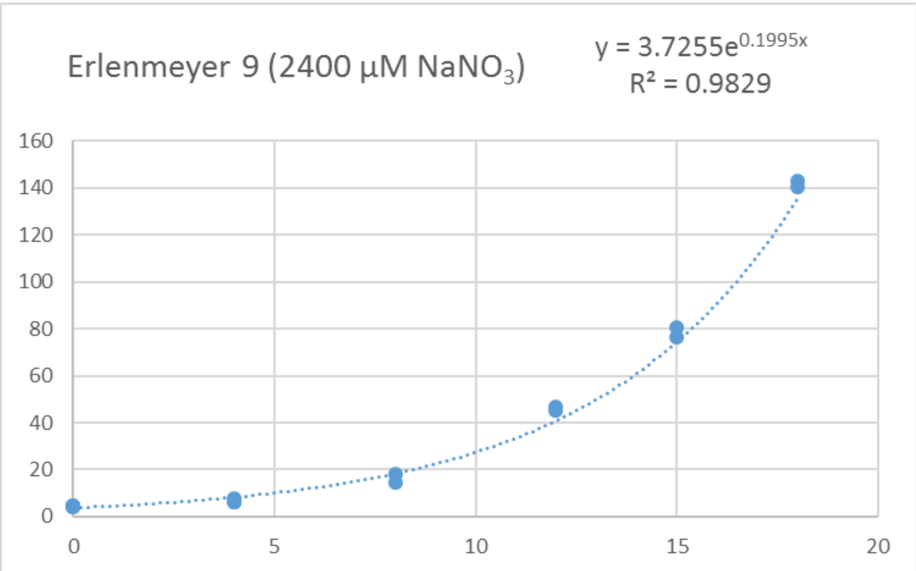

Erlenmeyer 4 (75 µM NaNO3)
y = 4.1818e0.1986x
R² = 0.9821
200
180
160
140
120
100
80
60
40
20
0
0
5
10
15
20


Erlenmeyer 5 (150 µM NaNO3)
y = 4.3264e0.2062x
R² = 0.9805
250
200
150
100
50
0
0
5
10
15
20

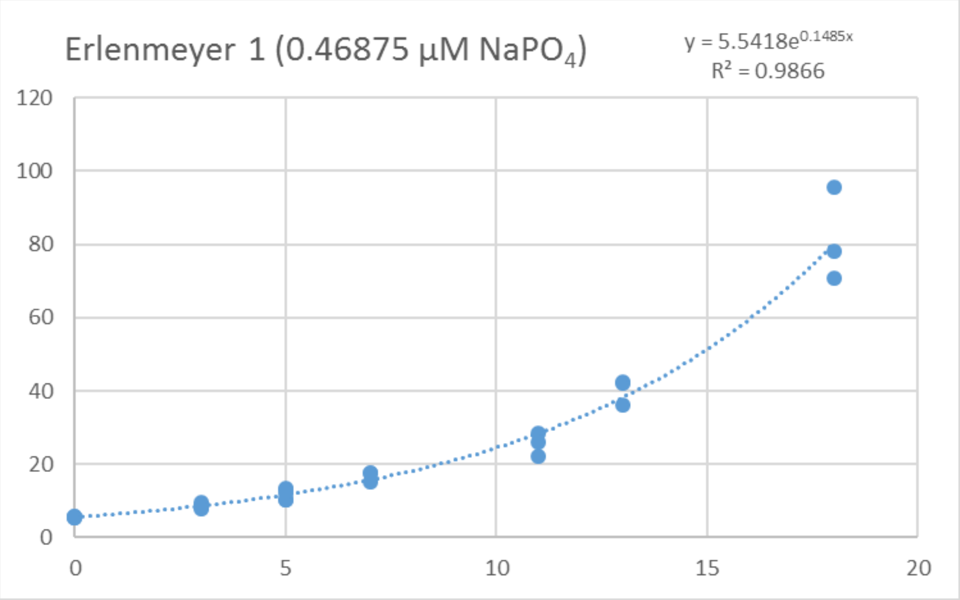

Erlenmeyer 6 (300 µM NaNO3)
y = 4.1535e0.1965x
R² = 0.9842
180
160
140
120
100
80
60
40
20
0
0
5
10
15
20

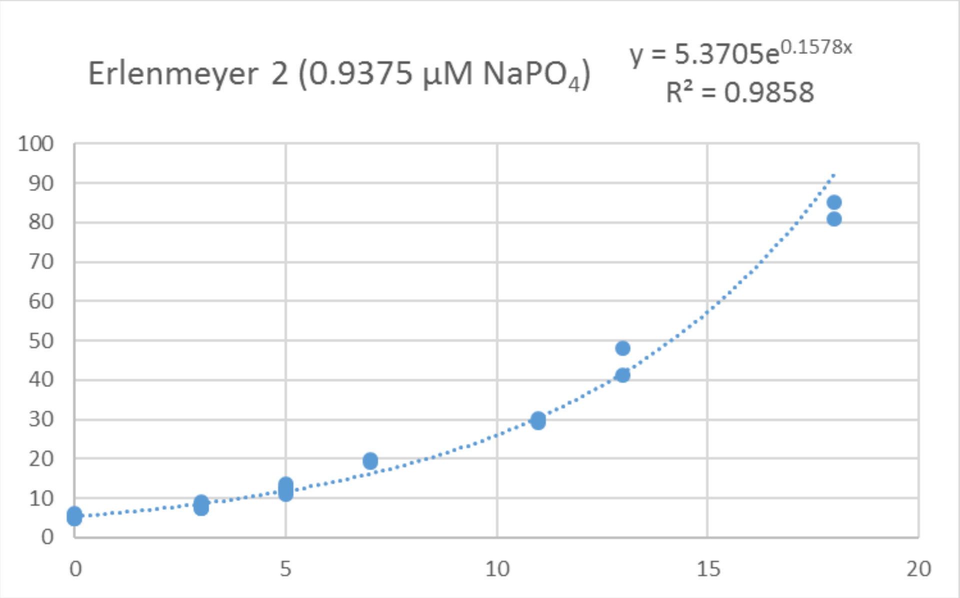

Erlenmeyer 7 (600 µM NaNO3)
y = 5.3017e0.1816x
R² = 0.9822
160
140
120
100
80
60
40
20
0
0
5
10
15
20

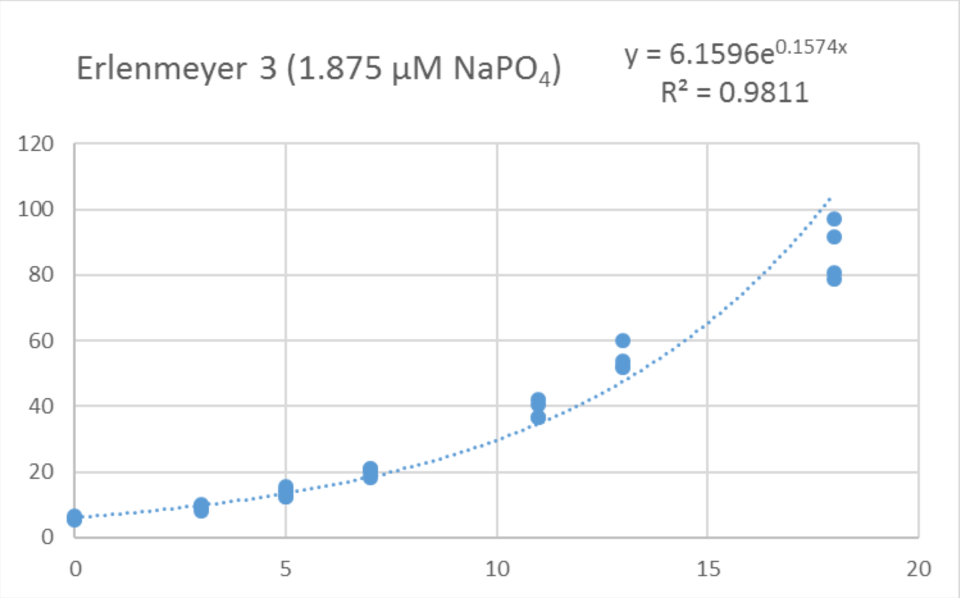

Erlenmeyer 8(1200 µM NaNO3)
y = 4.1866e0.1736x
R² = 0.9546
140
120
100
80
60
40
20
0
0
5
10
15
20

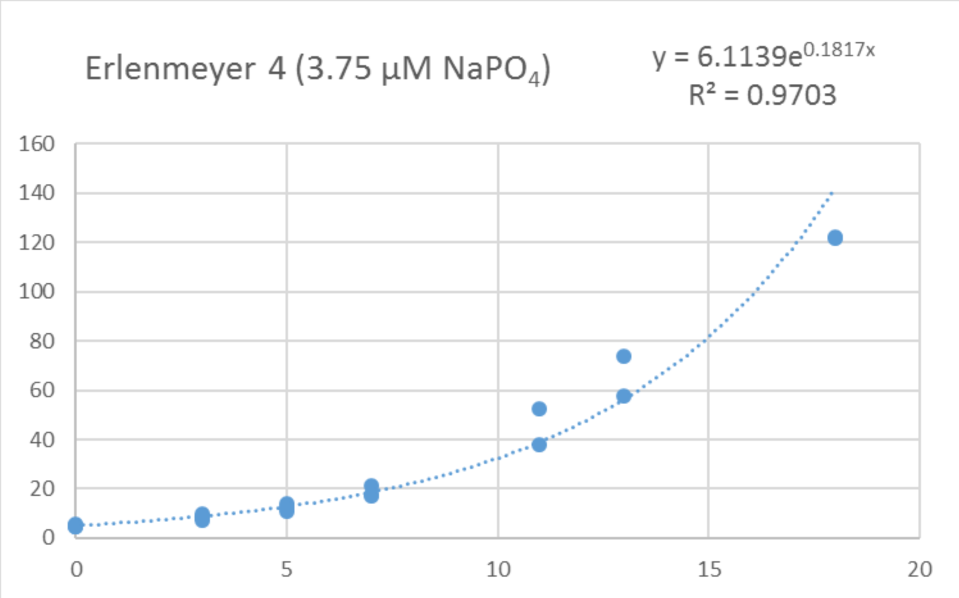

Erlenmeyer 9 (2400 µM NaNO3)
y = 3.7255e0.1995x
R² = 0.9829
160
140
120
100
80
60
40
20
0
0
5
10
15
20

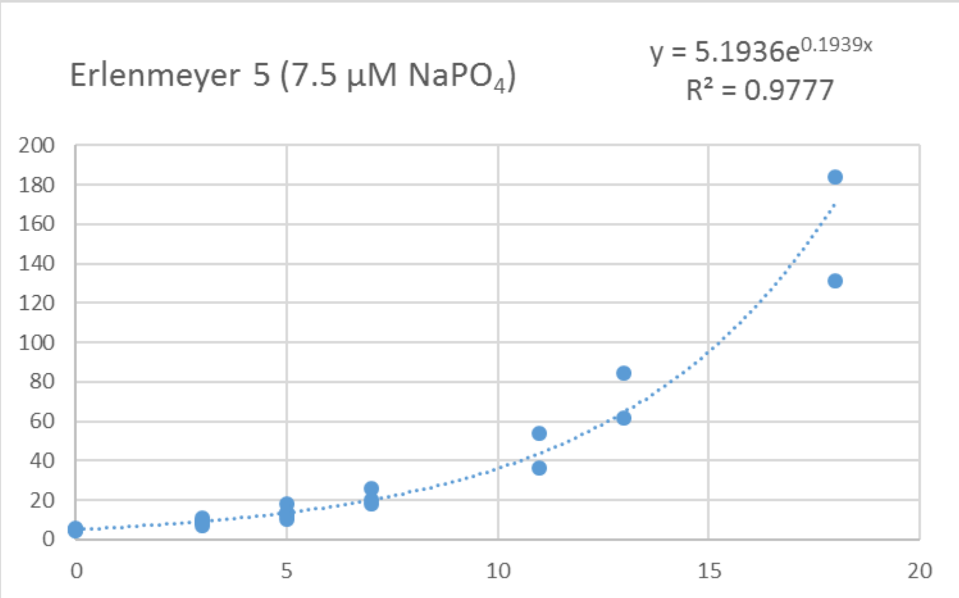
Appendix IV: RGR Curves per Erlenmeyer Flask ($NaPO_4$)

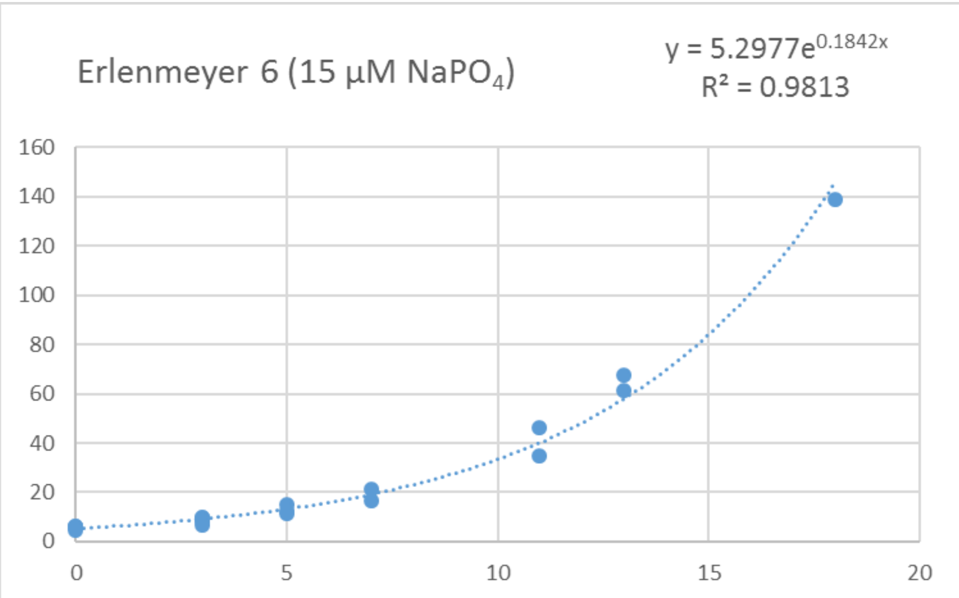


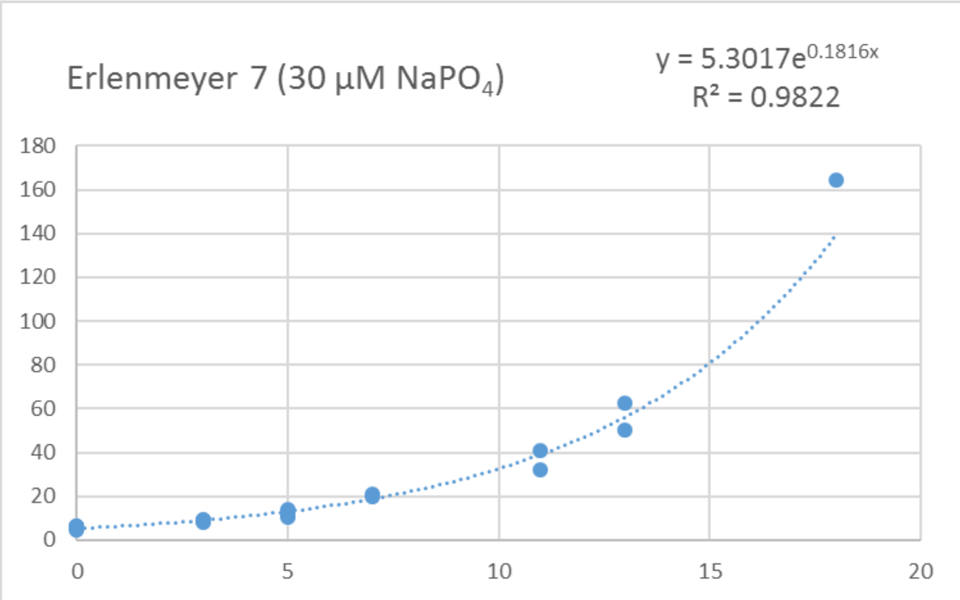


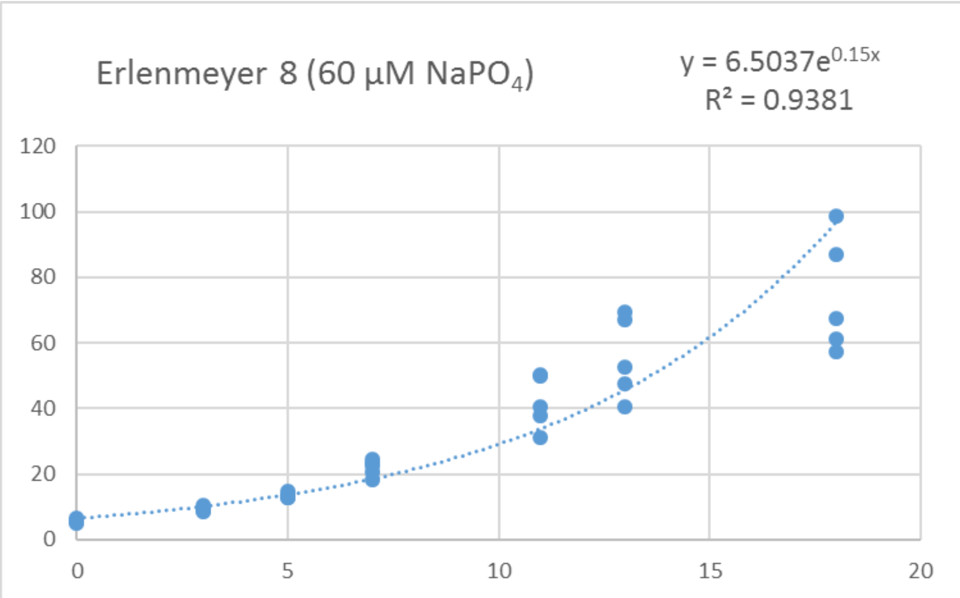


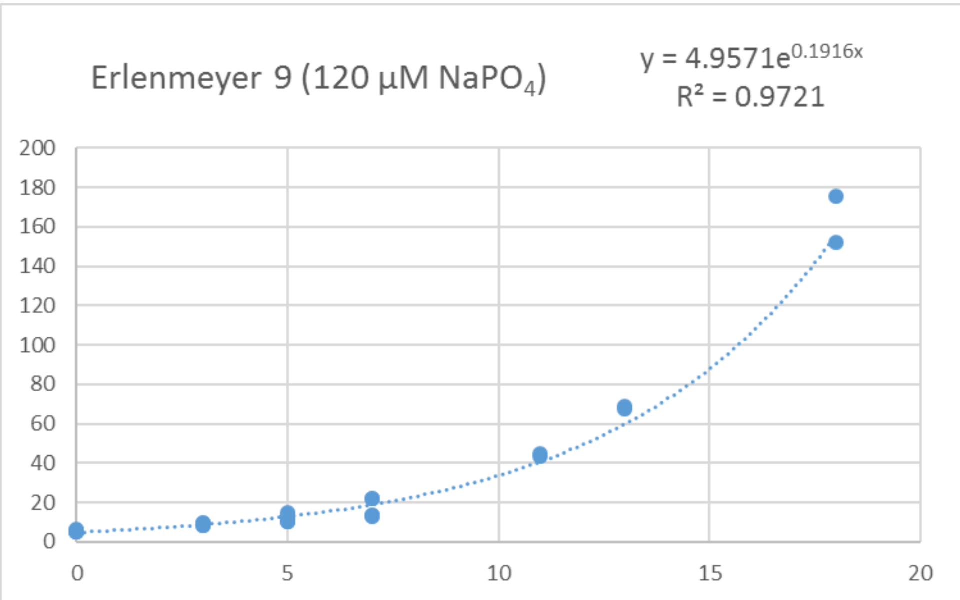

Erlenmeyer 4 (3.75 µM $NaPO_4$)
$y = 6.1139e^{0.1817x}$
$R^2 = 0.9703$
160
140
120
100
80
60
40
20
0
0
5
10
15
20


Erlenmeyer 5 (7.5 µM $NaPO_4$)
$y = 5.1936e^{0.1939x}$
$R^2 = 0.9777$
200
180
160
140
120
100
80
60
40
20
0
0
5
10
15
20


Erlenmeyer 6 (15 µM $NaPO_4$)
$y = 5.2977e^{0.1842x}$
$R^2 = 0.9813$
160
140
120
100
80
60
40
20
0
0
5
10
15
20


Erlenmeyer 7 (30 µM NaPO4)
y = 5.3017e0.1816x
R² = 0.9822
180
160
140
120
100
80
60
40
20
0
0
5
10
15
20


Erlenmeyer 8 (60 µM NaPO4)
y = 6.5037e0.15x
R² = 0.9381
120
100
80
60
40
20
0
0
5
10
15
20


Erlenmeyer 9 (120 µM NaPO4)
y = 4.9571e0.1916x
R² = 0.9721
200
180
160
140
120
100
80
60
40
20
0
0
5
10
15
20