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The influence of nutrient availability, salinity, temperature and the underwater light climate on the growth of seaweed

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Abstract

The goal of this thesis is to analyse the literature on the effects nutrients, salinity, temperature and the underwater light climate (intensity and colouring) on seaweed growth, weight, morphology, metabolism and composition. Nutrients have great impact on seaweed growth and composition. In the literature was found that there is a preference for the form of the nutrient (i.e. NH_3, NH_4^+, NO_2^-, NO_3^-) as well as the ratio in which they are available. There is still much research to be done to find the favoured nutrient form as well as the uptake kinetics, N:P ratios and nutrient fluxes seaweeds prefer. The salinity also influences seaweed growth and composition, but this is mostly in extreme environments. The extreme salinity of <5 PSU is not likely to occur in most situations and therefore it should not be a research priority, but optimal salinity can lead to increased growth. The water temperature is of greater concern and is a valid research field because there are certain ranges each seaweed species can endure and optima in which they thrive. The underwater light climate is a highly understudied field because there is no optimal light intensity studied yet, but there is found that a higher light intensity has a positive effect on growth and metabolism but no negative effect on the photosystem. Furthermore, the light colouring is also in the start phase, but it has shown to have effect on growth. In their study was suggested that a ratio between red and blue should be studied in the future. Lastly in the discussion is emphasized that all these abiotic factors need further research and more experimental research is needed to provide information which can be useful in production and models for seaweed cultivation. This way seaweeds become more commercially interesting and matrixes including several species of seaweed can be formed to provide an overview for researchers as well as companies interested in seaweed cultivation.

Contents

Introduction

Global food consumption is increasing because of human population growth which leads to a shortage of foods. As a result of the latter the western world shows a growing interest in the alternative food sources like salt water macroalgae known as seaweed (Holdt & Kraan, 2011). Cultivation of offshore produced macroalgae is a promising food source because it does not compete with food crops for arable land or potable water (Buschmann et al., 2017; Ross et al., 2018) Additionally seaweed are a good way for mitigation of climate change and are good for treating eutrophic waters which could otherwise suffer great biodiversity losses (Buschmann et al., 2017). The seaweeds are divided in three major groups, known as the green (*Chlorophyta*), brown (*Phaeophyta*) and red seaweeds (*Rhodophyta*). This division is based on differences in colour, habitat and morphology as well as chemical composition (Lobban & Harrison, 1994; Pomin, 2011; Wiencke & Bischof, 2012). Currently the leading producers of seaweeds are China and Indonesia with 23 million tons in 2014 which is 98% of total seaweed production. They mostly produce *Saccharina japonica* Areschoug (food), *Undaria pinnatifida* Harvey (food), *Porphyra* spp. (food), *Eucheuma* spp. (carrageenan), *Kappaphycus* spp. (carrageenan) and *Gracilaria* spp. (agar) (Buschmann et al., 2017).

Seaweeds are not limited to this and can be used in biofiltration and Integrated Multi-Trophic Aquaculture (IMTA) to neutralize the nutrient concentrations. Afterwards the produced seaweeds can be used in products like food, fertiliser, high-value products (carrageen, agar, etc.), or biofuels (Buschmann et al., 2017; Holdt & Kraan, 2011; Thomas & Kim, 2013). To reach the full potential of seaweeds cultivation and nutrient neutralization more research is needed in the effects of the most important abiotic factors which are nutrient availability, salinity, temperature and the underwater light climate. The latter factors are often broadly different for each species of seaweed and there are more robust species which can handle greater variation then other species (Lobban & Harrison, 1994; Wiencke & Bischof, 2012). Because of these differences it is interesting to analyse the already described findings and point out the research that should be prioritized.

This thesis will therefore evaluate research that measures the effect of abiotic factors on the growth, weight, morphology, metabolism and composition of the seaweed. This will be accomplished by analysing literature and figuring out the current knowledge on each abiotic factor separately. Afterwards the findings of this literature study will be used to discuss what information is currently missing to optimize usage of seaweeds. Finally, my analyses of the literature will be used to identify where experimental research should focus on to ensure efficient technological progress in the future.

Nutrients

Seaweeds are photoautotrophic organisms and are one of the primary producers found in salt and brackish waters. They synthesize organic material from inorganic nutrients like dissolved inorganic nitrogen (DIN) (i.e. NH_3, NH_4^+, NO_2^-, NO_3^-) and dissolved inorganic phosphorus (DIP) (i.e. PO_4^{3-}) along with light and CO_2. The produced organic material can then be eaten by other organism in the ecosystem or it can be used in foodstuff and other products, but the nutrient requirement of seaweed differs between species and it has only be determined for a select number of species.

Recently Ross et al., (2018) has published a study about the nitrogen uptake by the seaweeds *Cladophora coelothrix* Kützing and *Cladophora parriaudii* C.Hoek. In the introduction of their article is already mentioned that their study is intended to provide more information on the uptake rates of nutrients in these seaweeds for use in waste water treatment (WWT). WWT can be specified as the effect of seaweed to reverse the eutrophication caused by terrestrial runoff or produced by other sea products like fish cultivation. Eutrophication can have a harmful effect on the biodiversity when dead zones are formed by microalgae and are therefore of ecological and political concern because they want to add to the information of WWT. They are less interested in the surge uptake of the seaweed. The surge uptake is the ability of a seaweed to take up nutrients at an accelerated rate after their nutrient supply was exhausted, but WWT has a continuous supply of nutrients which means the surge up take is less relevant. They were more interested in the continuous or metabolic uptake of the seaweed and which nutrient form (NH_4^+,NO_2^-, NO_3^- and urea) was preferred, because this can differ per WWT. This kind of studies are seldom found because there is a lack of publicly available data on the influence of nutrient regimes in regards to nutrient form, concentration and N/P ratio on macro-algal growth, N uptake, and biochemical composition (Ross et al., 2018). After testing the effect of different concentrations and N forms on *C. coelothrix* and *C. parriaudii*, they found that the N form was of great influence. There was a preference found towards NH_4^+ and secondarily urea was taken up, but urea also enhanced the take up of NO_2^-, NO_3^- which were not preferred. However, the greatest influence was from the concentration of N in the medium, this greatly influenced the final composition and growth. They do recommend more research on different seaweeds to find species that are robust, flexible, and can simultaneously utilise multiple carbon and nitrogen forms.

Another recently published study is from Lubsch & Timmermans (2018), in this study they researched the uptake kinetics, storage and N:P ratios of *Ulva lactuca* Linnaeus. This study used laboratory controlled conditions to test *U. lactuca* in different N:P ratios and different concentrations to observe the surge and metabolic uptake as well as the ideal N:P ratio for maximum increase in surface area. They found the optimal N:P ratio to be 30:1 with a storage capacity of P from 0.73 ± 0.13 µmol × cm^{-2} and N 22.9 ± 7.0 µmol × cm^{-2}. The metabolic uptake for P was found to be 0.07 ± 0.04 µmol × cm^{-2} × d^{-1} and for N 2.3 ± 0.9 µmol × cm^{-2} × d^{-1} and the surge uptake rates were found to be 10 times as high in P and 5 times as high in N uptake. These uptake rates are important to know for implementation of a seaweed in production process. Because in production as well as nature nutrient availability can differ greatly and the ability of a seaweed to deal with these different nutrient fluxes seems to be important. However,

this is one of the few studies who accurately measure uptake kinetics and N:P ratios of a seaweed in laboratory conditions.
The importance of a seaweed to deal with nutrient fluxes is described by Tyler, McGlathery, & Anderson, (2001). The goal of this study was to investigate the influence of *U. lactuca* on the nutrient concentrations and the sediment-water column fluxes of dissolved organic nitrogen (DON), urea and dissolved inorganic nitrogen (DIN). In this study it is important to know the difference between concentration and flux. The concentration can be measured in µmol per m^{-2} on a point in time, while the flux is the transfer of molecules measured in µmol per m^{-2} d^{-1} from the sediment to the water column. In their study they show that *U. lactuca* mediates the DIN and urea fluxes from the sediment to the water column and increases the DON flux to the water column in shallow temperate waters. In their study is also explained that degradation of the seaweeds will occur when nutrient fluxes are too low. This is a problem which has not been studied often and it would be interesting to know how much nutrient flux is needed to sustain and grow *U. lactuca* and other seaweeds.

From this chapter can be concluded that there is a lack of studies on seaweeds regarding the form of nutrients they prefer as well as the uptake kinetics, N:P ratios and fluxes needed to grow and enhance the composition of different seaweeds. Future research should therefore focus on collection of this kind of data for a diversity of seaweeds to enable comparison of their beneficial futures.

Salinity

Seaweeds are found in a wide range of salinity gradients. The salinity of water is often measured in practical salinity units (PSU). This is an electrical conductivity measurement to estimate the ionic content of the water. The salinity in open sea is quite constant between 33-37 PSU and is often lower and less constant in coastal waters or closer to the polar regions, because of runoff through rivers, rain and ice adding to the seawater. Seaweeds can live in the littoral and sublittoral zone, they are protists and are protected by a layer, which protects them from harmful salinity and acidification of the sea. They are therefore able to survive in these conditions.

An ecological study performed by Schubert, Feuerpfeil, Marquardt, Telesh, & Skarlato, (2011) links seaweed diversity to salinity in the Baltic sea. During the study 10 sampling sites were used with a salinity ranging from 3.9 to 27 PSU. The sampling sites in this study were on the same latitude and there was no eutrophic terrestrial runoff which limits the effect of abiotic factors on the study. This study shows a clear trend between higher salinity and the number of species present in the sample. In the discussion is also explained that below 5.4 PSU one species normally became dominant. From this study can therefore be concluded that higher salinity normally leads to more species diversity and more species probably prefer higher salinity gradients. The study does have an ecological interest and does give a general idea about the influence of salinity on seaweed, it does not satisfy the knowledge of seaweed regarding their flexibility in salinity ranges per species.

Another study researching the effect of salinity on seaweed is the study of Martins, Oliveira, Flindt, & Marques, (1999). In their study they researched the effect of salinity on the growth rate of the seaweed *Enteromorpha intestinalis* Linnaeus. They noticed that *E intestinalis* normally had higher growth rates during spring unless there was more precipitation during winter and spring. The latter was the reason they tested the effect of a range from 0 – 32 PSU on the growth rate. They measured the growth rate in specific growth rate μ per day for *E intestinalis*. They performed the study outside with influences of the environment, but used the average salinity found over the test period. In their study was found that salinity did have impact on the growth rate of *E intestinalis*. They found 0 PSU to be lethal in hours and 3 PSU the critical low limited while higher PSU above 28 PSU also showed reduced growth. They were not as critical as 3 PSU or lower. The higher growth rates were between 10 – 22 PSU and optimally at 17 – 22 PSU. From this study can be concluded that there is an effect of salinity on the seaweed which can in extreme cases cause the seaweed to slow growth and in fresh water to die. The other conclusion that can be made is that at least *E intestinalis* does have a broad salinity range where he can survive and salinity can be a critical factor for optimization of seaweeds growth.

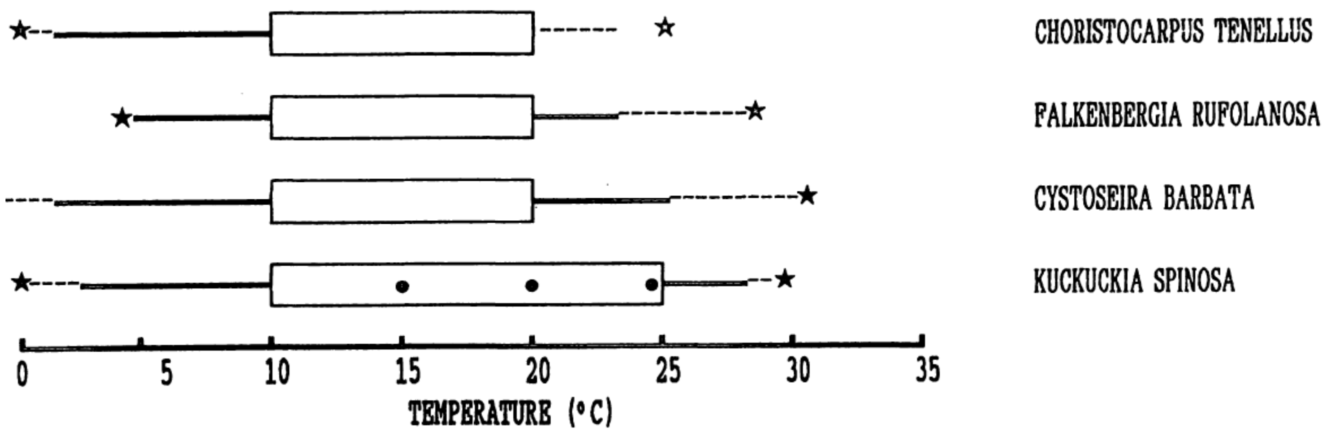
The study of Angell Alex R., Mata Leonardo, Nys Rocky, Paul Nicholas A., & Buschmann A., (2015) mostly focused on the effect of salinity on the amino acid content of *Ulva ohnoi* M.Hiraoka & S.Shimada (holotype). During this study they used a salinity range of 10 -60 PSU with increments of 5 during 21 days and measured the quantity of the total amino acid (TAA), the quality of the amino acids and methionine (MET) of the seaweed. The TAA was determined by calculating the grams of amino acids found in 100 grams of dry weight. The quality was determined by ratio in which they were found and the MET was the grams of methionine found per 100 grams and was determined separately because it is often the limiting amino acid. From this study the first result was that *U. ohnoi* was not able to withstand a salinity < 20 PSU for a longer time. They also found that TAA was negatively linearly related to growth and showed a weaker nonlinear relationship with salinity. This is an indirect effect of salinity on the quantity of TAA because salinity slowed the growth rate. They did find a direct effect on the quality of the protein where increasing salinity led to increase of proline, tyrosine, and histidine but a decline in alanine. The most limiting amino acid MET did not show a linear relationship and instead peaked at 40 PSU and was lower at higher/lower PSU levels. From there study could be concluded that there are direct and indirect effect of salinity on amino acid content of seaweed. But there should be taken in consideration that the essential amino acid MET did not grow with higher salinity. This study shows that salinity does have effect on amino acid content but mostly through the influence on growth rate and salinity can be manipulated to enhance the production of certain amino acids.

Over all can be concluded that proper salinity does have a complimentary roll in production and can be a limiting growth factor and does influence the amino acid levels. The literature research also showed that salinity is mostly limiting during low salinity and seaweeds can generally withstand a broad range of salinity. The salinity should mainly be considered when there is major fresh water runoff in a region or much rain in an area where the salinity will drop close to fresh water levels. This should not be underestimated because most eutrophic areas will be near coastal areas where salinity is fluctuating and can be low. From the literature can be seen that these fluctuations can be lethal if they would persist for a few hours. However, at this point salinity should not be prioritized in research because most seaweeds seem to be stable in a broad salinity range.

Temperature

The rising temperatures and acidifications are both results of climate change and are affecting the global seawater temperatures. In this chapter the influence of temperature on seaweeds will be the main subject. Extreme temperatures can be lethal for seaweeds and moderate changes in temperature can lead to reduced growth and reproduction. There has already been described that ocean warming may cause approximately 25% of the seaweeds to die off in the next 60 years (Wernberg et al., 2011). Because of the latter reasons it seems important to investigate the effects of temperature chance on seaweeds.

One of the studies investigating the effect of temperature on seaweeds is the study of Orfanidis (2009). In his introduction he already describes the temperature ranges a seaweed can endure and that temperature ranges can be used to map geographical distribution. During his study his objective was to investigate the temperature response of four seaweeds (*Kuckuckia spinosa* Kützing (holotype), *Choristocarpus tenellus* Zanardini (holotype), *Cystoseira barbata* Stackhouse (lectotype) and *Falkenbergia rufolanosd* Harvey belonging to the warm-temperate Mediterranean-Atlantic group (Orfanidis, 2009). The results of his study showed that *K. spinosa* grew optimally between 15 - 25 °C and sufficiently at 10 to 25 °C. The lowest survival temperature tested over 8 weeks was 3 °C and the upper temperature tolerance was 28 °C. but sexual reproduction only took place at 20 and 25 °C. *C. tenellus* grew optimally at 20 °C and sufficiently at 10 - 20 °C. the lowest survival temperature was 2°C and the highest temperature was 26 ºC but sexual reproduction was still undetermined. *C. barbata* grew optimally at 15 °C and sufficiently at 10 to 25 °C. The lower temperature tested for 8 weeks was 3 °C, when they tested the upper temperature they found that an 8 week exposure to 30°C the seaweed was able to regenerate. The seaweed staid sterile throughout the whole experiment. *F. rufolanosa* had a sufficient growth rate between 10 – 20 °C. The lower survivable temperature was 5 °C and the upper temperature was 26 °C But sexual reproduction did not take place for at least 12 weeks. An overview of the results from this study can be found in Figure 1.



Temperature intervals limiting growth, reproduction and survival of the investigated macroalgae. The symbols in the diagram are defined as (——) survival without injury in the interval (------) regeneration from injured thallus during the post-cultivation in the interval; (★) lethal temperature; (□) sufficient growth in the interval; (●) asexual reproduction.

Figure 1 Result overview of the study of Orfanidis, (2009) The study of Orfanidis, (2009) concluded that this information can be of invaluable importance in understanding the geographical boundaries of the seaweeds. Secondarily this information is of critical importance for the understanding of seaweed usage, because in this study is made clear what the ideal conditions are for these seaweeds and at which point the seaweeds struggle. Of course, there should be taken in consideration that this are seaweeds from warm-temperate Mediterranean-Atlantic which means they probably have similarities in temperature range and flexibility. However, this study shows that seaweeds are quite resilient to fluctuating temperatures but there are optimum temperatures, which can greatly enhance growth. Also, can be seen that the flexibility differs between species and it is important to discriminate this before using a seaweed for production.

The study of Nejrup, Staehr, & Thomsen, (2013) studied the effect of temperature and light dependent growth and metabolism of the red seaweed *Gracilaria vermiculophylla* Ohmi and compared this to two native seaweeds. During their study they performed two experiments, they first tested for interactive effects of light and temperature on relative growth (μ), using 0, 9, 16, 34, 80 and 225 μmol photons m^{-2} s^{-1} light levels crossed with 5, 10, 15, 20, 25 and 30°C temperatures. During the second test an investigation was performed on the ability of *G. vermiculophylla* to adjust its metabolic rates to short-term incubation temperatures which stated at 14 °C and were daily adjusted by 5 °C to match 10, 15, 20 or 25°C. After the short term temperature adjustments they measured the associated changes in the tissue concentration of chlorophyll a, nitrogen and carbon (Nejrup et al., 2013). The results found during the second test were compared to *Fucus vesiculosus* Linnaeus (lectotype) and *U. lactuca* which are both native species but have different archetypal K and r growth strategies. The results of experiment one showed that light dependency of growth differed significantly between experimental temperatures. The optimal light and temperature combination was 20°C and 225 μmol photons m^{-2} s^{-1}. The results of experiment two showed that the temperature had a great impact because 20 °C was optimally and there was a significant difference in growth between 10 – 20 °C. they also observed that *U. lactuca* had a similar growth ratio to *G. vermiculophylla* but there were differences found in the interaction of the physiology parameters (C, N, C : N and chlorophyll a). When comparing temperature dependencies*, G. vermiculophylla* had a weaker response to higher temperatures than *U. lactuca* regarding somatic growth, photosynthesis and respiration. Furthermore, the calculated optimum temperature for the photosynthesis at the temperature at which the algae had long-term acclimated, was more or less the same for the two species (Nejrup et al., 2013). In contrast, the biochemical analysis of thallus content grouped *G. vermiculophylla* together with *F. vesiculosus* (Nejrup et al., 2013). The relevance of this study to this present thesis is that the temperature did influence the light regime and internal composition of the seaweed, which means that the effect of temperature on seaweeds are not only limited to growth, but also have effect on their light usage and internal composition.

The articles of Nejrup et al., (2013) and Orfanidis, (2009) are two articles that show the impact of temperature on the growth, internal composition and metabolic management of seaweeds. There is only a limited number of studies that measured the effect of temperature on seaweeds. More studies should be performed to know which species are usable in each temperature climate.

Underwater light climate

The underwater light climate can be separated in light intensity as well as light colouring which both impact seaweeds. The underwater light climate can be broadly different depending on depth and clarity of the water. Seaweeds are often adjusted to this and therefore have different colouring. The red macroalgae is normally found in deeper areas where only blue light can penetrate and therefore the red macroalgae reflects red light. Also, the clarity of the water makes difference because opaque water is only barely penetrated by light and therefore primary producers must live close to the surface to be able to catch some light. Not all seaweeds are able to life in these conditions and some seaweeds will have a fitness benefit over the other, depending on the water penetrability and depth of the seafloor.

The effect of light intensity on the seaweed *Ulva rigida* C. Agardh (holotype) was investigated by Yıldız & Tiryaki, (2017). During their experiment they used two different light regimes 55 µmol photons $m^{-2}s^{-1}$ and 100 µmol photons $m^{-2}s^{-1}$. Afterwards the photosynthetic performance, growth rate, pigment content, total protein and nitrate reductase activity were measured. Their results showed that the relative growth rate, nitrate reductase activity and chlorophyll-a concentrations of *U. rigida* decreased significantly at 55 µmol photons $m^{-2}s^{-1}$ light regime in comparison to 100 µmol photons $m^{-2}s^{-1}$. From this result could be concluded that light intensity does not influence the photosynthetic performance in this range, but light intensity does have an increasing effect on nitrate reductase which catalyses the reduction of nitrate to nitrite and increases the growth of *U. rigida*. The latter results show enhanced growth under higher light intensity but less protein per gram of seaweed. The former results already show that light intensity is of great importance in seaweed growth and thus, research regarding the effect of light intensity on seaweed seems to be important.

During a similar experiment performed by Chen, Zou, Zhu, & Yang, (2017) there was also tested for an effect of light intensity on seaweed *Gracilaria lemaneiformis* Bory (lectotype). During this study they also checked for two light intensities 135 µmol photons $m^{-2}s^{-1}$ and 45 µmol photons $m^{-2}s^{-1}$. Additionally they tested the effect on acidification on this seaweed but this won't be addressed during this thesis. The results about acidification can be found in their study (Chen et al., 2017). Their results showed that a higher light level enhanced algal growth and decreased water loss but reduced amino acid accumulation. These results are very similar to the results of Yıldız & Tiryaki, (2017) but are tested on a different seaweed and have minor difference in light intensity. This result leads us to belief that higher light intensities generally have a positive effect on growth of seaweed. These results do not yet offer us the ideal light intensity for the growth of the seaweeds, which would be interesting for seaweed cultivation.

There is a study found on the effect of light colouring on a seaweed. This effect is described by Takahide et al., (2017). In their study they measured the effect of intermittent light and continues light as well as the colour red, blue and white light on the gametophytes and sporophytes of *U. pinnatifida*. From there results could be seen that there was no marginable difference found between intermittent light and continues light. This is an unexpected effect because phytoplankton and land plant favour intermittent light. Even lettuce does favour intermittent light but only in high frequencies (100 Hz or more). They did make two notable comments on this subject, they mentioned that their light intensity for the gametophytes might be too high after further investigation. Secondly, they mentioned that the absence of a day night cycle may also have a promoting effect on the growth of *U. pinnatifida*. For the light colouring they first explained that former studies already suggested that *Eisenia bicyclis* Kjellman (holotype) and *Scytosiphon lomentaria* Lyngbye (holotype) gametophytes grew well under blue LED (Dring & Lüning, 1975; Murase, Abe, Noda, & Suda, 2014). During their own study they found that both gametophytes and sporophytes grew well under white light and poorly under blue and red light. Blue and red light resulted in smaller leaves and deformities in sporophytes and less gametophyte forming. Because green light is not absorbed by the blade it is suggested that a ratio between red and blue light should be found. They found that red and blue light of almost the same strength are promoting health in *U. pinnatifida* but an ideal ratio is yet to be found. The later study of Takahide et al., (2017) shows that at least the light colouring does have a great effect on growth efficiency of the seaweed. They were not yet able to determine the ideal ratio which would be important for the enhancement of seaweed growth. The day night cycle and intermittent light and continues light factor do seem to be a work in progress. all these factors are only determined for a few seaweeds while there can be seen that there are great impacts on seaweed growth.

Discussion & conclusion

During this thesis we discussed a variety of literature regarding the effect of abiotic factors on growth, weight, morphology, metabolism and composition of seaweeds. Most of the literature used is not focused on the same seaweed, which has the pro that this thesis paints a general picture of abiotic effects on seaweed. The con is that the specific data provided by the literature cannot be used to suggest interactive effects between abiotic factors or give a conclusion which abiotic parameters are so to say 'Best'.

From the chapter Nutrients was concluded that there is still a lot of research to be done on the nutrient form a seaweed prefers as well as the uptake kinetics, N:P ratios and fluxes needed to grow and enhance the composition of seaweeds. This is mostly important for seaweed cultivators on land. They produce seaweeds in basins and are able to have some control on abiotic factors in contrast to sea cultivation. for instance, because the cost production efficiency should be as high as possible for land cultivators. Therefore, it is necessary to be able to calculate the exact nutrient concentration, ratios and fluxes to have high growth and have the lowest possible nutrient input. For the use in eutrophication treatment it is more important to know which seaweed does have the highest uptake rates, because there will obviously not be a nutrient limitation. In this case the seaweeds are more exposed to other abiotic factors which have greater impact on growth, weight, morphology, metabolism and composition. Still there is a lot of research to be done in this abiotic factor. First there should be more research done on which seaweeds prefers which form of nutrient to find a good de-eutrophication seaweed. Secondly uptake kinetics and N:P ratios must be described for more seaweeds. Currently only a handful of seaweeds have this data available while it is described that the right ratio is of importance in cultivation. Also, the effect of fluxes on seaweeds is yet to be described, there was no study found which particularly describes this subject. Although the study of Lubsch & Timmermans, (2018) did show the Internal storage capacity which shows that seaweeds do have a storage of nutrients. From the surge uptake and internal storage capacity can be calculated how long and how often a seaweed needs nutrients. From this study we can't see the effect on long term nutrient fluxes on the seaweed. When those factors are known and established for several seaweeds it can be used to compare the nutrient requirements of seaweeds.

In the second chapter was concluded that Salinity can have a major effect on seaweeds, but it must be extreme salinity gradients to harm most seaweeds. These salinity gradients are only found in coastal areas or polar waters because of the input of fresh water. Salinity will not likely pose a threat to land cultivation of seaweed although specific salinity can enhance seaweed production. For cultivation in coastal waters the lower salinity could pose a problem for reaching the highest production rate. Therefore, it will be interesting to know how great the influence is of salinity on the growth and composition of different seaweed species. It would also be interesting to know what the effect is of salinity fluctuation on seaweeds. In places where there are clear raining seasons the salinity can fluctuate greatly in relative short time. Therefore, it is important to know these places what the phenotypic plasticity is of the seaweeds.

During the third chapter the effect of Temperature on seaweeds was considered. This effect of temperature has great impact on the growth, internal composition and metabolic management of the seaweeds. There are not a lot of studies performed on the effect of temperature on seaweeds.

Therefore, the evidence for the effects must be further investigated. Temperature seems to be specific for the seaweeds and there are differences in temperature gradients that seaweeds can endure. There is also an optimal temperature range in seaweeds, which can be important for the cultivation "on land". These temperature ranges are also important in natural cultivation at coastal areas, but in natural cultivation the seaweeds are more likely to be selected for the region then the other way around. Furthermore, the effect of temperature fluctuation has not been investigated yet while it is a valid circumstance in coastal areas. Because energy plants, which use cooling water and the effect of environmental temperature of which the influence are greater in run of streams, do probably fluctuate the temperatures in coastal waters.

In the last chapter about the Underwater light climate there was made a distinction between light intensity and light colouring. In the literature this was also described as light quantity and light quality subsequently. In the search for this literature it was apparent there were only a small number of studies found. At this moment there were no studies found describing the optimal light intensity or light colouring. The studies that were found showed that higher light intensities do have a positive effect on seaweed growth and metabolism. Which is to be expected of a primary producer but does not yet answer the question what the upper limit is of the light intensity. This could be of great value in coastal water cultivation where in sunny areas measures could be taken to prevent burning of the leaves. This is already done in land-based seaweed cultivation by obscuring the light because seaweeds are there grown in shallow waters. Also, light colouring has been proven to harvest results in terrestrial production, so further investigation in the light colouring is also necessary. The study discussed in this thesis was still inconclusive about the optimal light colouring. They did make it probable that there is a ratio between blue and red to be found which is optimal for a seaweed species. However, the light intensity should be one of the first concerns to study, because there is little known while it can have great positive impact on production.

It is also important to see the big picture for future seaweed usage in cultivation and eutrophication treatment. To make it commercially interesting to start growing seaweeds it is of great importance to gather data of all these abiotic variables to be able to make an educated estimation of the stable production of the seaweeds. To do this mathematical models have already been put forward and one of the more recent models is of Lehahn, Ingle, & Golberg (2016). In their study they made a mathematical model for *Ulva* spp. metabolism and growth. They calculated the algal growth rate (μ) as a function of light intensity (I), temperature (T), salinity (S) and dissolved nutrients (nitrate and phosphate) and respiration rate. This model took in to account the seasonal fluctuations and was based on a farm which would be 400 km offshore and 100 m deep. With a surface area of ~10^8 km^2 the model predicted an output of 10^{11} dry weight tonnes $year^{-1}$. The model did predict much fluctuations and is still only a model of which the input data must be extended. The model is surely on the right track and an even more complex model could be used to make a matrix for several seaweed species to make it easier available for commercial use. Also, the model can be enriched by taking in account the effect of seaweed density, acidification, light colouring and grazers. Which are not all discussed during this thesis but do have effects on seaweed growth.

Finally, from this thesis can be concluded that although al abiotic factors do influence the seaweed growth, weight, morphology, metabolism and composition. Some abiotic factors are more urgent to investigate then others. From this thesis can be seen that mainly light and nutrients have the greatest impact on seaweeds and seem to greatly limit the cultivation of seaweeds if not sufficient. Nevertheless, al abiotic factors have need for more investigation. Furthermore, models are important to make a clear overview of all the data currently available and it would be favourable to make a matrix of seaweed species were researchers can add their data too.

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