Influence of salinity in grow and photosynthetic activity in different *Ulva* germlings and optimization of selective breeding

BACHELOR OF SCIENCE

Biology

By Anja Sawicki

(Student ID:4381581)

Submitted to

University of Bremen

Faculty of Biology / Chemistry

First Supervisor: Dr. Laurie C. Hofmann (Alfred Wegener Institute)

Second Supervisor: Prof. Dr. K. Bischof (University of Bremen)

Bremen 30.01.2022

ALFRED-WEGENER-INSTITUT
HELMHOLTZ-ZENTRUM FÜR POLAR- UND MEERESFORSCHUNG

Universität Bremen

Abstract

"Mak-Pak" or "Mak-Pak Scale-Up" is a project for the industrial implementation and use of a packaging concept consisting of macroalgae in the food sector. The aim is to develop a biodegradable, sustainable and edible packaging material from macroalgae as a dosage form and for "out-of-home" consumption. For this, different algae strains will be studied and the best conditions for high productivity will be researched. The growth and the complex reproduction cycle play a major role in biomass production. This thesis intends to answer the question how different salinity treatments (10,15,20 and 30 PSU) affect the growth and photosynthetic activity of germlings from *Ulva lacinulata, Ulva californica* and *Ulva linza*. By measuring the photosynthetic activity and growth, optimization of the experimental method was developed. The different salinities of 10,15,20 and 30 PSU showed significant differences in grow area and relative growth rate. The photosynthetic activity differed, but not significantly. Differences in the tolerability of different salt levels have been shown between species. The method of experimental setting was optimized and discussed. Based on the optimized method the question if it's possible to use this method to differentiate between phases of the life cycle (between Gameto- and Sporophytes) was explored for *Ulva californica*. In cultivation selective breeding plays a major role. Using the photosynthetic activity to differentiate between lifecycle stages seemed inconclusive and is discussed.

Contents

Introduction

The pollution problem

Every piece of plastic ever made still exists today. For the fact that plastic does not break down easily, we make far too much of it and a significant amount of our plastic waste ends up in the environment. In 2016 Germany generated around 38 kilograms of plastic packaging waste per person. Only in Luxembourg (50.5kg), Ireland (46.2kg) and Estonia (42.2kg) the consumption in Europe was even higher (BUND, 2019). On global average each person produces 0.74 kilograms of waste per day and the amount increases related to wealth. Fatally, plastic bags for transport in particular are just used 25 min on average. By 2015 more than 8.3 billion tons of plastic waste were generated globally. Of this only about 9 percent were recycled and 79 percent landfilled or disposed of in the environment (10 Scary Facts About Plastic, 2021). Unfortunately, it takes about 450 years for a plastic bottle to dissolve into microplastic components. For a fishing line it is said to be as long as 600 years. Since plastic waste is not biodegradable it causes massive damage to our nature. For example, according to a study, a northern fulmar has an average of 34 plastic pieces in its stomach, weighing 0.31 grams (BUND, 2019). The consequences of plastic in our meals and its distribution in environment are largely unexplored. For example one study of 38 different examined mineral waters showed microplastic particles in all of them (Schymanski, D. et al 2018). The need for the development of alternative and sustainable solutions is evident.

Algae as a resource

Algae represent a good and versatile resource with a lot of potential for example as packaging alternatives. The algae industry as a sector will gain importance in the future also due to its sustainability. In the past the Algae *Ulva* has been used for various applications: food, fuel, aquaculture, cosmetic products, colouring dyes, therapeutical and botanical applications (Dhargalkar, V. K. ,2006; Kelly and Dworjanyn, 2008;Notoya, 2010; Battacharyya et al., 2015). Those past trends grew due to the lack of resources. The advantage of *Ulva* is biodegradable, non-toxic, non-polluting and non-hazardous to humans (Dhargalkar, V. K. ,2006). Therefore *Ulva* is used for nutritional strategies, disease management and water efficiency (Arioli, Mattner & Winberg, 2015). Other than that intensive agriculture and aquaculture by

using *Ulva* could compensate lack of food. Moreover, from a biotechnological point of view, *Ulva* could be used as a biofilter or as an organic biostimulator (Guttmann et al., 2018; Paulert et al., 2021).

Unfortunately, the algae industry is negatively affected by pollution, so that some wild species contain high contaminants (Bouga & Combet, 2015). Especially for wild algae compliance for consumption seems more difficult than under land based conditions (Ferdouse, Holdt, Smith, Murúa & Yang, 2018). Arsenic, cadmium and iodine are the biggest problems here, whose levels exceeded the maximum levels in most studies. Aquaculture systems are a good solution to avoid contaminants and to produce continuous quality in food or algae materials like contact materials or food packaging. In addition, the production of biomass can be better regulated and higher growth rates could be realized. This makes it lucrative due to certain advantages. Land based farming also allows full or partial recirculation to be performed, making the entire technique cost effective (Balar, N. B. & Mantri, V. A. ,2019).

Mak-Pak Scale-Up

As a nice pollution solution is the project: "Mak-Pak" or "Mak-Pak Scale-Up" which is funded by the German Federal Ministry of Food and Agriculture. Thus, a biodegradable, disposable, sustainable and edible packaging material is to be developed from macroalgae, as a dosage form and for "out-of-home" consumption.

Processes need to be optimized and scaled up for the production. Different algae strains are investigated and the best conditions for high productivity are researched. Algae well suited for aquaculture systems are selected. Growth as well as the complex reproduction cycle play a major role which will be investigated within the framework of the project. The preselected strains will be cultivated with agricultural partners in land protected culture facilities and -if necessary- processes will be optimized there too (Alfred Wegener Institute, 2021).

Raw material will be produced from extracts of the macroalgae intending to represent a health benefit for the consumer in addition to the packaging purpose. The design will be developed and further tested by Nordsee GmbH. In cooperation with Bremerhaven University of Applied Sciences, Hengstenberg GmbH & Co. KG and Pulp Tec GmbH & Co. KG, the suitable raw materials from the algae material will be identified and refined. This is done by extension

with additive, sustainable, bio-based materials from adjacent agricultural and food industries and optimization of technical processes (Hochschule Bremerhaven, 2021).

The consistent standardized productivity and quality as well as a traceable control option play a major role in order to be able to guarantee a rapid market launch. From laboratory setting to an industrial scale there is still much to discover.

Aims and Hypothesis

The growth and photosynthetic activity of different *Ulva* species might be affected by the salinity of seawater (Chen, B. & Zou, D. ,2015). In order to define the best salinity conditions for *Ulva* spp. to growth in a land based system with artificial seawater, it is neccessary to know what the best conditions for each species are. For the project, the optimal growing conditions need to be determined and scaled in order to grow a large amount of biomass without compromising quality. As part of the 'Mak-Pak Scale-Up" project, this thesis focuses on the cultivation of three *Ulva* species germlings with four different salinity treatments to answer the following questions:

- How does different salinity treatments affect the growth and photosynthetic activity of *Ulva* spp. germlings?
- Which species grows better in the lowest salinity?
- What are the best conditions to grow each species?

The first hypothesis: The growth and photosynthetic activity differs between the salinity treatments during the experiment of *Ulva Californica, Ulva linza* and *Ulva lacinulata* germlings.

To figure out these questions the experimental set up was optimized to find the best settings in a pre-experiment. The data was collected in a second experiment. Both set ups are discussed in the end.

Based on the optimization, a third experiment with the germlings could be realized, referring to the life cycle. The background is to find a way of selective breeding. It is intended to answer following questions:

- Is it possible to use the imaging PAM (a pulse-amplitude modulated fluorometer) to differentiate between phases of the life cycle (between Gameto/Sporophytes)?
- Is the photosynthetic activity a good way to determine reproduction?
- What is a good method to induce reproduction?

By determining this questions, the life cycle should be better understood and controlled in order to be able to induce, control and scale artificial breeding with this method.

The second hypothesis: The photosynthetic activity varies on reproductive days and between the gameto-and sporophytes.

Methods

Experimental set ups

The algaes for the experiment were different *Ulva* strains.

Ulva linza, a tubular species, was collected in Portugal in Lagoa de Óbidos on the 4th of January 2020 by Isabel Cardoso (Figure 1.1 A).

Ulva lacinulata, morphological with blades, was picked up at the same time and location (Figure 1.1 B).

Ulva californica was picked up in 1986 and cultivated for years at the Alfred Wegener Institute. It is a unipolar culture from Greek, Thessaloniki, provided by the Rocky Shore Section by Dr. Inka Bartsch (Figure 1.1 C).

The germlings where used for the experiment when the algae reproduced.

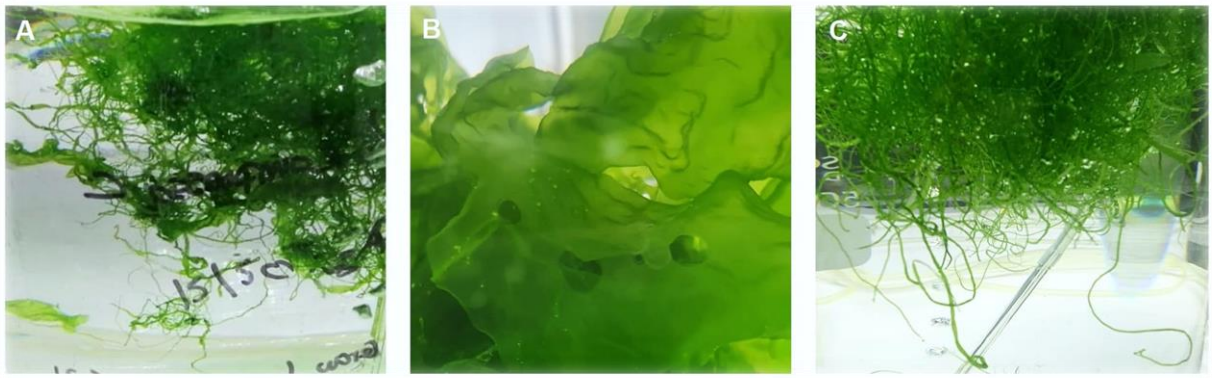

Figure 1.1 A) *Ulva linza* **B)** *Ulva lacinulata* **C)** *Ulva californica*
(Photos: Anja Sawicki, 2022)

First Experiment:

For the first experiment (as a pre-experiment) *Ulva Californica, Ulva linza, Ulva lacinulata germlings* where placed in four different salinity treatments: 10, 15, 20, 30 PSU.

For each treatment four replicates (48 samples) were placed in one Petri dish with 10ml of treatment water. The water was artificial seawater with added nutrients (½Provasoli solution: 10 ml sterile stock per liter of sterile 30 ppt. filtered seawater).

Simultaneously, control groups of 2 individuals with AWI seawater (natural seawater from Helgoland with 33 PSU) and artificial control (called “ZAF” ;33PSU with ½ Provasoli solution) were also provided with one germling per petri dish.

The light intensity was 30-40 µmol photons m^{-2} s^{-1} with a 16:8 daylight ratio and they were cultivated by 15°C.

The growth was documentation of photographically twice a week. The size was measured with a photo measuring software (Image J,v 1.52 Rasband, 2021). The dishes were placed next to a 3x3 cm reference square on a LED tablet and photographed from above with a Canon Camera.

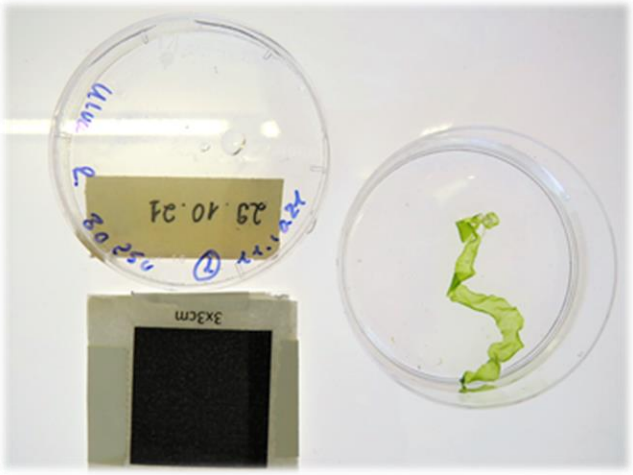

Figure 1.2 Experimental setting of photodocumentation with reference square. (Photo: Anja Sawicki, 2021)

The photosynthetic activity was determined by measurement with a pulse amplitude fluorometer (Imaging PAM (Heinz Walz GmbH, 2020)) twice a week. The water has been exchanged once a week. The experimental duration was 21 days with seven measuring days in total. Before the photosynthetic activity was measured, the germlings where covered for 10 min. for dark acclimation.

Second Experiment

The construction of the second experiment was similar to the first one. The settings for the fluorescence measurement were adjusted and the germlings were positioned to grow in well plates (12er Wells). Instead of positioning, photographing and measuring with the imaging PAM, each petri dish was now sufficient to position 12 germlings at one time. This allows a faster evaluation of the photos. Before the photosynthetic activity was measured, the germlings where also covered for 10 min. for dark acclimation.

Instead of four individuals per treatment, three were used, so that in the end 36 germlings per species were measured. In addition there were two controls groups per species in AWI natural seawater (33 PSU, from Helgoland, called “ASW”) and artificial seawater (also 33 PSU, called “ZAF” with ½ Provasoli solution added). The water of the treatments was produced like in the first experiment. The volume of the wells was smaller: 5 ml.

All other settings remained the same: temperature, light and light frequency. Water was changed once a week to avoid nutrient deficits. Furthermore, microscopic checkups of the germlings were performed weekly to detect any reproduction. The duration for the experiments was 21 days.

Figure 1.3 experimental set up of the second optimized experiment. In the Background the Petri dishes of the fist experiments are seen. (Photo: Anja Sawicki, 2021)

Third Experiment

For the third experiment only the species *Ulva californica* was used to differentiate between gameto-and sporophytes.

It was cultivated in a salinity of PSU 33 in AWI seawater, enriched with ½ Provasoli solution, in 15C° and constant light conditions like in the first experiment. Four well plates were used (this time six per plates). The volume was 10 ml per well and a total amount of 24 germlings were examined. Daily two thirds of the water was changed after the measurement to avoid nutrient deficits and inhibition of reproduction by germling produced reproduction inhibitors. The plates were acclimated for 30 min and after a 10 min dark acclimation measured by Imaging PAM (photosynthetic activity). After the first fluorescence measurement of the experiment, two well plates were subjected to heat shock. Well one and two were incubated for one hour at 25C°. Well three and four were incubated at 4C° for one hour. Subsequently, photosynthetic activity was measured again.

After one week, a second shock induction was performed. In this case, all the germlings were incubated in water-free conditions for one hour under normal temperature (15C°).

Every day, microscopic observations were carried out and identification was continued.

Data analysis and methods

The measurements of photosynthetic activity and Photos for the growth area were taken from the first day and repeated twice a week in experiments 1 and 2. In the second experiment, microscopic observations were also performed by biweekly.

For the third experiment photosynthetic activity was measured every day for each germling. Microscopic observations were also performed daily.

Growth of the experiment 1 and 2

For the total surface area of the germlings, the surface area (SA) was measured. The Petri dishes were placed next to a 3x3 cm reference square on an LED tray and photographed from above. Images were analyzed using Image J software (1.52 Rasband, 2021).

The photos were modified by changing contrast and pigment so that green parts became red. The outlines of the red objects were captured with special tools (Figure 2.1). The total surface area could be calculated with the help of further tools by scaling the quadrate area (Miller, L. ,2011). The surface area was doubled to calculate the total surface area (TSA) which is available for photosynthesis.

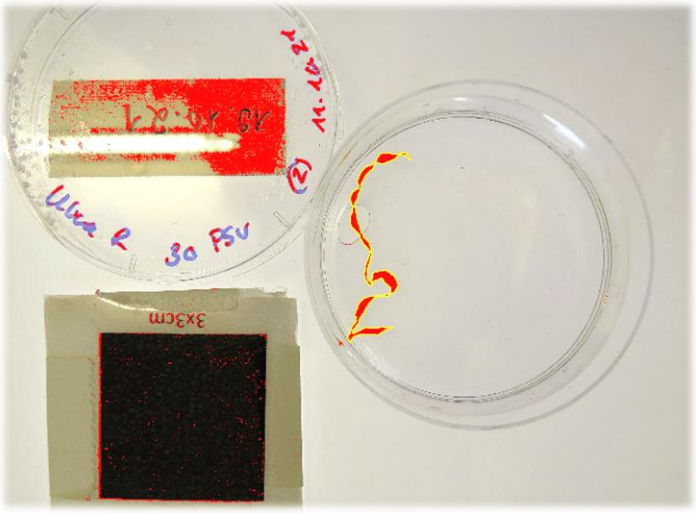

Figure 2.1 Contrast changing of green pigments to in Image J and outline of the germling area (yellow). (Photo: Anja Sawicki 2021)

TSA cm^2 = 2 * SA (cm)

The relative growth rate RGR expresses by what percentage the surface area increased compared to the previous sampling time. This is calculated as follows:

$$RGR = \frac{\ln(SA2) - \ln(SA1)}{t2 - t1} x100\%$$

Photosynthetic activity

Chlorophyll occurs in the form of a pigment-protein complex in photosystem II, photosystem I, and light-harvesting complexes (LHCs). The light energy absorbed by chlorophyll molecules can drive photosynthesis (photochemistry) and can be reemitted as heat or fluorescence.

These three processes do not occur in isolation but in competition with each other. Therefore the yield of chlorophyll fluorescence emission provides information about the quantum efficiency of photochemistry and heat emission. The proportional competition between these processes allows us to determine the efficiency of photosystem II. In this context photochemistry serves as the energy and reduction force for CO2 assimilation.

Since chlorophyll fluorescence is a measure of the light remitted by photosystem II, any ambient light can interfere with the measurement of fluorescence (Lawson, T. 2013). Therefore, all samples were acclimatized to darkness for 10 minutes prior to measurements and measured in the dark. Figure 2.2 shows a typical fluorescence curve. *Fo* is the minimum fluorescence level at which the reaction centers are open even in the dark. A short saturating light pulse leads to the formation of the maximum possible fluorescence yield (*Fm)*. During this pulse, the reaction centers are closed (Lawson, T. 2013). Chlorophyll-a fluorescence reaches its maximum *Fm* when the photosynthetic electron transport chain is saturated and all additional absorbed light energy is reemitted.

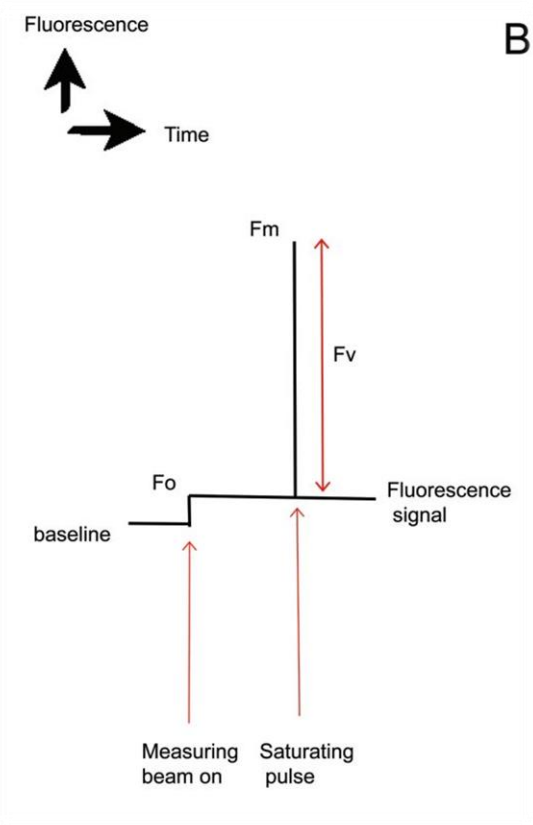

Figure 2.2 Fluorescencecurve (Lawson, T. 2013)

To find *Fv*, the *Fm-Fo* has to be calculated, which was detected with the Imaging PAM (fluorometer).

Fv/Fm is the maximum quantum efficiency of the PSII photochemistry and is often used as an indicator of plant and algal health (Higo et al.,2017).

Finally, it can be calculated as follows: $\frac{(Fm-Fo)}{Fm}$

In the experiment, *Fo* and *Fm* was emitted by the pulse amplitude modulated (PAM) fluorometer: "Imaging PAM" (and the associated ImagingWin v2.41a software (Heinz Walz GmbH, Effeltrich, Germany)).

For the first experiment, the settings were chosen as follows for all algae and where optimized during the experiment : Saturation pulse: 5, Gain: 4, Intensity: 3, Light curve points:10, Minimum light range: 0,2-0,3.

For the second experiment, the settings were optimized for each species:

Ulva linza: Saturation pulse: 1, Gain: 4, Intensity: 4, Light curve points: 10, Minimum light range: 0,06-0,08.

Ulva Californica: Saturation pulse: 1,Gain: 4, Intensity: 6, Light curve points: 10, Minimum light range: 0,06-0,08..

Ulva Lacinulata: Saturation pulse: 5, Gain: 3, Intensity: 5, Light curve points: 10, Minimum light range: 0,2-0,3.

In each experiment three measurement points were set per sample in all experiments.

In the third experiment *Ulva californica* was measured by the same settings.

Reproduction identification

Daily microscopic examination was performed to determine whether reproduction had started in experiment 3. All reproductions were noted. Swarmers could be picked up for identification with a pipette under the microscope (Zeiss AG, Germany invert darkfield microscope). Using Lugol's solution on a separate microscope slide, swarmers where prevented from swimming (they stopped moving their flagella's) and counting the flagella's was

Figure 2.3 Swarmers contrasted by darkfield microscopy (yellow). (Photo: Anja Sawicki, 2021)

possible to identify gametes or spores. Photos were taken for documentation (Figure 2.4).

A sample with possible swarmer's was exposed to a light source in a pipette tip, darkened with aluminum foil to increase the density of swarmer's. Because the gametophytes seem to follow the light, a particularly large number of swarmer's should be concentrated at the tip of the pipette to increase the density for identification of gamets. Spores would concentrate in the dark. A separation was tried out with this technique.

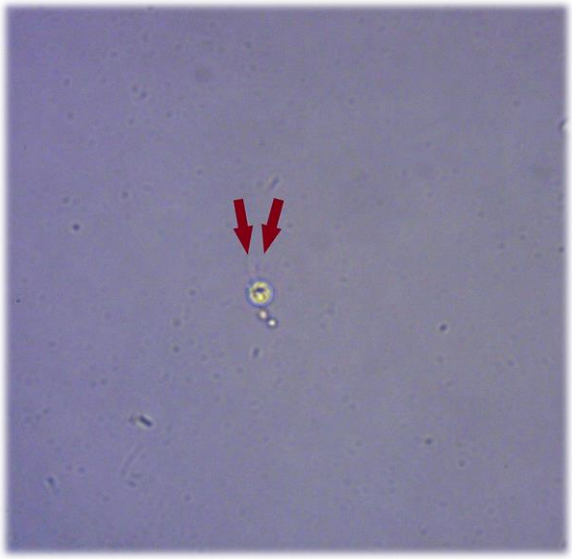

Figure 2.4 Observed gamet with two flagella. (Photo: Anja Sawicki, 2021)

Statistical Analysis

All calculations were prepared and organized in Microsoft Excel (Microsoft Corporation, Redmont, USA). Statistical analyses were performed using R- Studio software (version 4.0.5 (R Core Team, 2021)).
The normal distribution of the data was checked with histograms and Shapiro-Wilk test. Variance homogeneity or variance between groups were analyzed using Levene's test of equality of variances. If a significant effect of treatment or duration (days) on the measured variable could be shown, a post-hoc TukeyHSD test was performed for pairwise comparison of the groups.

Two-way mixed ANOVA was applied to compare the mean values of treatment groups by the factors salinity and duration (days). These tests where used for the total surface area and fluorescence measurements in the salinity experiment.

Two-way repeated measure ANOVA was applied to analyze differences of photosynthetic activity over the duration (days) and individuals (to differ between gameto and sporophytes).

A significant difference between groups was assumed when the p-value was less than 0,05. All calculations were summarized in tables A1-A9 in the appendix.

Results

Growth

Ulva linza

Over the period of time, it became clear that the algae achieved a different growth depending on the treatment. The growth between treatments differed significantly ($F_{5,12}$= 4,29; p =0,018) and day ($F_{5,60}$= 140,55; p < 0,0001) for *Ulva linza*. The interaction of day and treat was not significant. A significant difference among treatment groups occurred at day 14. Here, algae from treat 30 differed significantly from treat PSU 10 algaes (p=0,0104). PSU 10 15 and 20, as well as the two control groups differed among themselves but not significantly. On the last measurement day, all groups differed from each other but not all significant. Group 30 was significantly different from 10 (p=0,00063). Group 20 and the two control groups were also significantly different from treatment group 10.

Growth was observed in all treatments (Figure. 3.1) and the largest algae of treatment PSU 30 reached a maximum total surface area of 3,049±0,320 cm² followed by the artificial sea water (ZAF; control group) with 2,8±0,333 cm². In contrast, PSU 10 only reached 1,477±0,237 cm², less than the half. The algae grown in PSU 20, as well as those grown from AWI seawater (ASW) and ZAF (artificial seawater) were relatively similar in TSA (total surface area).

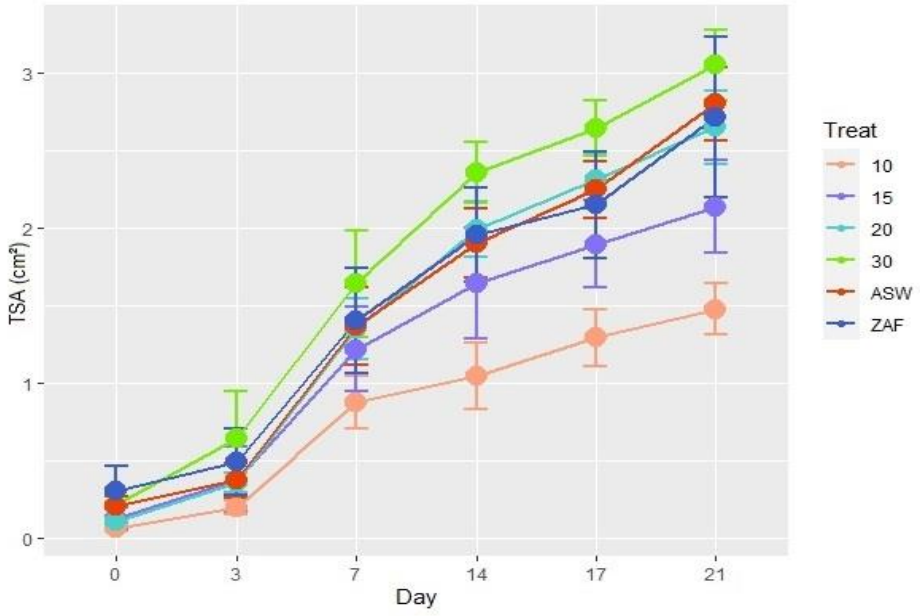

Figure 3.1 Total surface area in cm² over a duration of 21 days of *Ulva linza*. Errorbars indicate the standarderror.

Ulva californica

The analysis showed a significant difference between the groups of salinity treats ($F_{5,12}$= 31,85; p <0,0001) and days ($F_{5,60}$= 159,99 ; p < 0,0001) in terms of growth. Growth was observed in all Treats (Figure 3). On the last day (21), PSU 30 was significantly different from PSU 15 and 20. The algae of treat PSU 30 reached a total surface area of 2.110±0.387cm². The algae grown in AWI Seawater reached the largest maximum of 3.201±0.247, followed by the artificial Seawater (ZAF). Both grew at a salinity of PSU 33. PSU 20 achieved the lowest growth of 1.408±0.242 and was not big different from PSU 10 and 15 (Figure 3.2).

In contrast to the other *Ulva* species, *Ulva californica* grown in AWI Seawater was able to build up the most biomass within the duration.

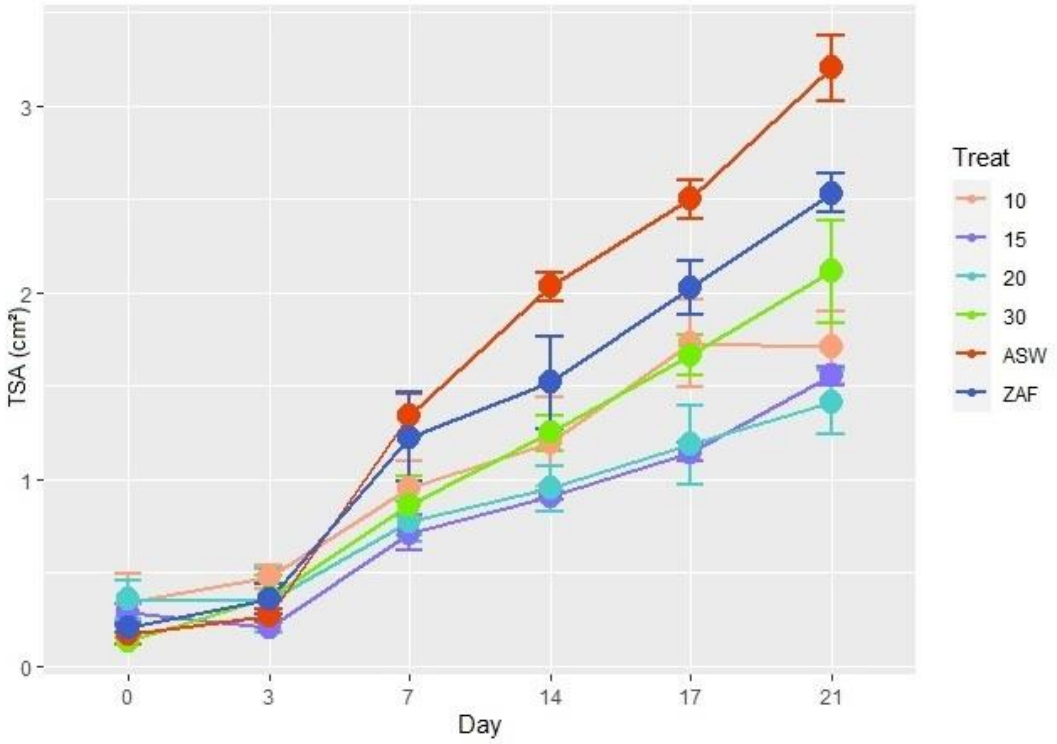

Figure 4.2 Total surface area in cm² over a duration of 21 days of *Ulva californica*. Errorbars indicate the standarderror.

Ulva lacinulata

The analysis revealed a difference in growth of the treats conditioned by salinity ($F_{5,12}$= 70.025; p=6.4*10-8) and duration (days) ($F_{5,60}$= 151,99 p=5,47*10-6).

On day 0, the germlings did not differ significantly from each other. From day 7 on, PSU 30 differed significantly from PSU 10 (p=0,00033). All other groups differed in size, but still not significantly.

On day 14, PSU 30 (p=0,0000001) and 20 (p=0,033) differed significantly from group 10. In addition, PSU 30 differed significantly from also 15.

On the last day, all differed significantly except PSU 15 from PSU 10.

The algae from PSU 30 developed the largest total surface area with a maximum size of 1,657±0.321cm². In contrast PSU 10: 0,099±0.017cm² (figure 6). The difference of PSU 30 to the two control groups was not significant.

Visually, it was seen that the blades in treat PSU 10 defragmented and looked pale at the end (Figure 3.4). Photosynthetic activity was still carried out. PSU 15 also looked pale and fragmented. Compared to the other algae, this species achieved the lowest growth.

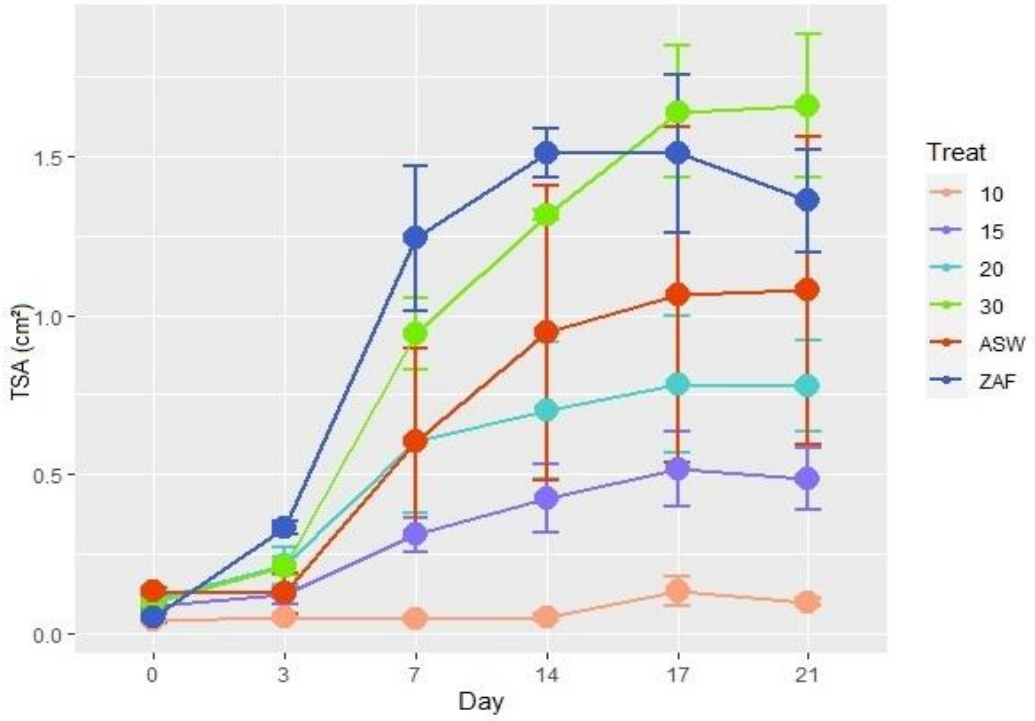

Figure 5.3 Total surface area in cm² over a duration of 21 days of *Ulva lacinulata*. Errorbars indicate the standarderror.

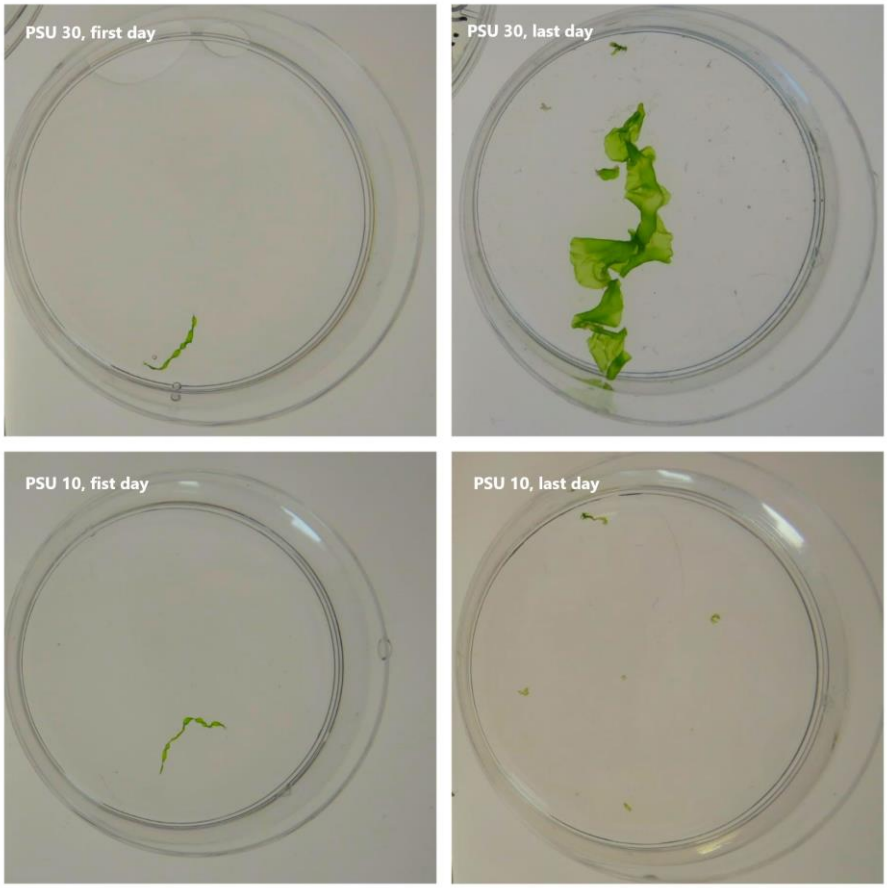

Figure 3.4 Comparison of observed growth for *Ulva lacinulata*. PSU 10 grew at the beginning and fragmented to the End. In comparison, this PSU 30 grew well**.**

Photosynthetic activity

Ulva Linza

No significant difference on photosynthetic activity between the salinity treatments was calculated (p=0,079). The difference between the treatments and days was significant (p=0,000266). Photosynthetic activity on the first day was very low and increased until the third day. Afterwards it decreased continuous every day (Figure 7). Unexpectedly, a decrease on day 14 was seen but not significant in treatment PSU 10. At the end the photosynthetic activity decreased from the highest in PSU 10 on day 3 0,7158±0,012 (*Fv/Fm*) to 0,537± 0,0133. Also the controls decreased in a slow way similar to the different treats. The highest photosynthetic activity on the last day was measured in the PSU 30 condition: 0,0515±0,0737, followed by PSU 15, 10 and 20 as the lowest. However, it seemed, at the beginning, the lower salinity conditions had a higher photosynthetic activity than higher salinities, comparing Day 3 to 14, but not significantly.

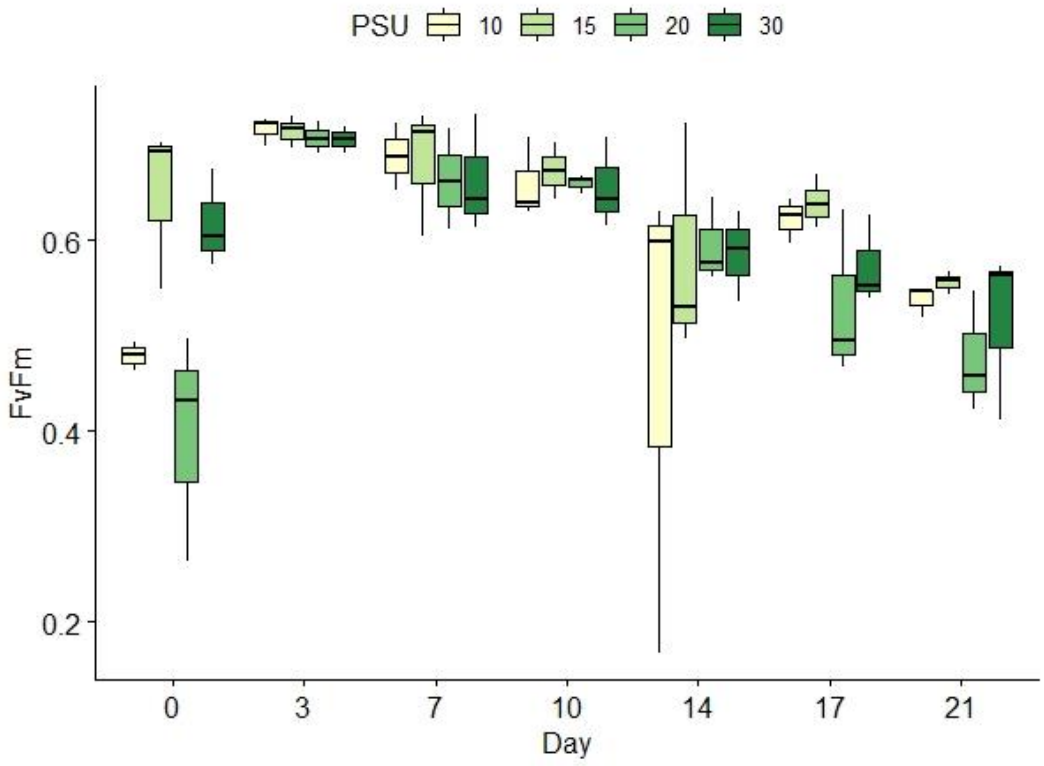

Figure 3.5 Maximum efficiency of photosystem II (Fv/Fm) over a duration of 21 days and different salinity treatments: PSU 10, 15, 20 and 30 of *Ulva linza*.

Ulva californica

The difference between the treatments and duration was significant ($p=3,77*10^{-11}$). Salinity had no significant influence on photosynthetic activity between the treatments. However, especially the first day of measuring seemed to differ enormous in contrast to the other days (figure 8). Over time a decrease of the photosynthetic activity was detected in all treatments. For example in PSU 10 from 0,670±0,0456 to 0,493±0,0459, measured as *Fv/Fm*. PSU 30 had the highest photosynthetic activity at the end and similar to *Ulva linza*, the lowest at 20.

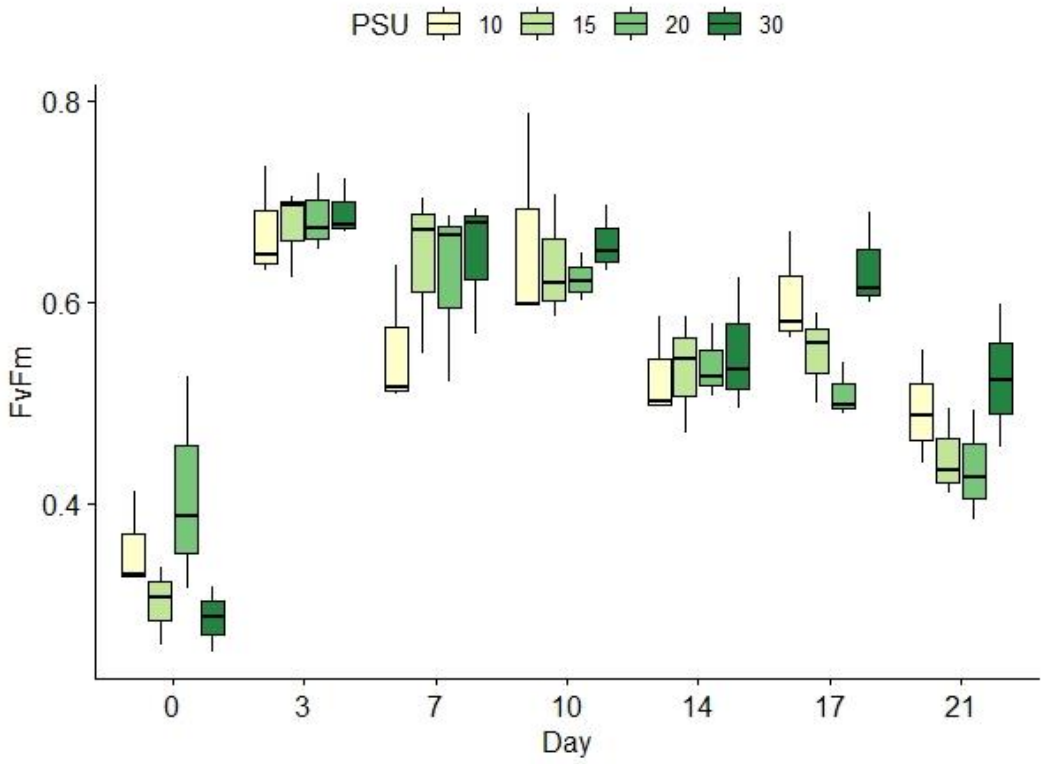

Figure 3.6 Maximum efficiency of photosystem II (Fv/Fm) over a duration of 21 days and different salinity treatments: PSU 10, 15, 20 and 30 of *Ulva californica*.

Ulva lacinulata

Salinity had no significant effect on photosynthetic activity between all groups. Only the days had a significant effect on photosynthetic activity (p=1,78*10-7) .

Inexplicably, PSU 30 from day 1 was significantly different from all the other treatments (figure 9). On day 3 there were no significant differences. Over time, a tendency for

photosynthetic activity to decrease can also be seen in all treatments (also the controls). At the beginning the photosynthetic activity of PSU 10 with 0,573±0,0614 (day 0) it decreased to 0,525±0,013. The AWI Seawater control had the highest value 0,541±0,128 at the end, followed by ZAF (artificial seawater) to PSU 10, 20, 30 and then 15 (lowest). However, the differences were not significant.

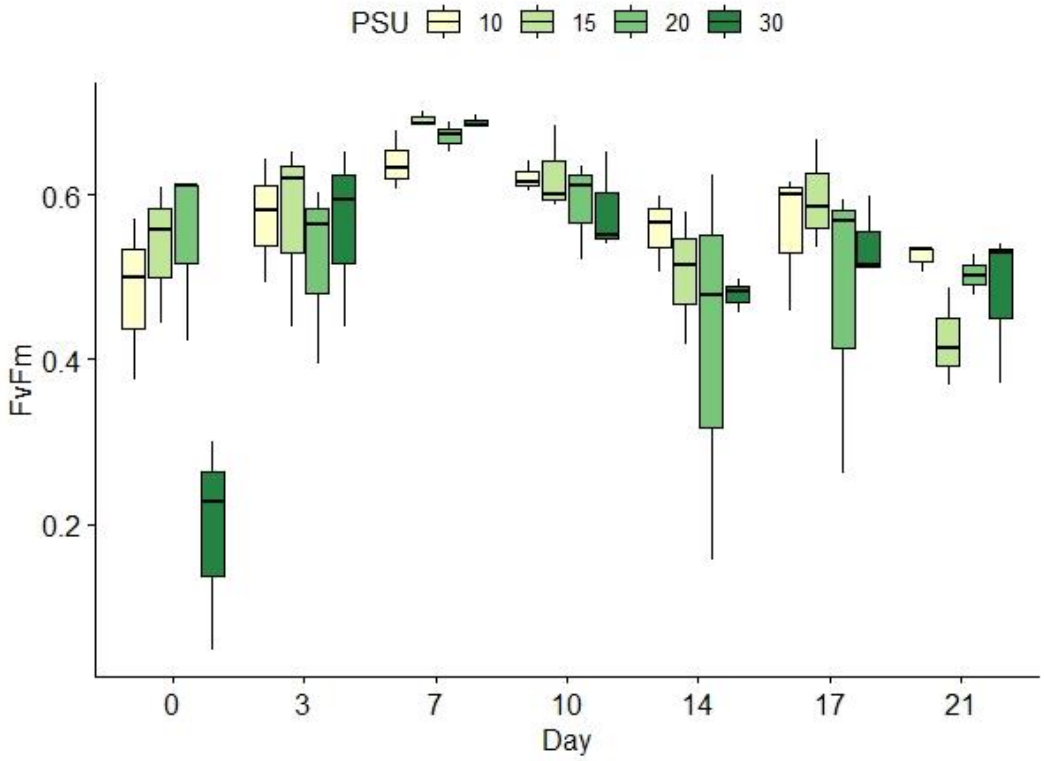

Figure 3.7 Maximum efficiency of photosystem II (Fv/Fm) over a duration of 21 days and different salinity treatments: PSU 10, 15, 20 and 30 of *Ulva linza*.

Life cycle experiment

With a invers darkfield microscope, pictures all the germlings after Lugos's solution treatment could be taken each day. Therefore, by focusing and using the Lugol's solution to interrupt the flagellar movement, it was always to see, that they all had two flagella.

The first swarmers were observed on 13.12, 7 days after the cold shock in plates 1 and 2. After the first temperature shocks it was observed that only a few swarmers were repeatedly released. The second shock, in which the germlings were left dry for an hour, resulted in mass reproduction events. So, it was observed that dry shock was the most effective way to increase reproduction enormously trigger. Also interesting is, that reproduction always happened between 10 and 12 am. The heat shock, treated in plates 3 and 4 developed swarmers later, when the dry shock was done. Well 3 showed few swarmers for the first time on 15.12.2021 and it seems that photosynthetic activity increased from 14.12.2021 to

15.12.2021 by some individuals. However, this also happened by non-reproducing individuals. It seems to increase after some reproductions and decrease in others e.g. well 1 reproduced on 14.12, resulting in an increase; in 1c neither decreased after reproduction. So it is not clear if increase or decrease of photosynthetic activity goes hand in hand with reproduction.

All fluorescence measurements of the germlings were evaluated. Special focus was on the reproduction days. Figure 3.8 shows the fluorescence measurements over time in the well plates 1,2,3,4. The photosynthetic activity increased or decreased on reproductive days. The differences between day values are significant: Well 1 ($p=5,43*10^{-9}$), Well 2 ($p=1,35*10^{-22}$), Well 3 ($p=3,46*10^{-22}$) and Well 4 ($p=3,71*10^{-9}$). Table 1 allows a directly comparison on reproductive days to photosynthetic activities in Figure 3.8. Differences between the individuals to compare between gameto-and sporophytes are not significant for all: Well 1 (p=0,047), Well 2 (p=0,033), Well 3 (p=0,254, not significant) and Well 4.

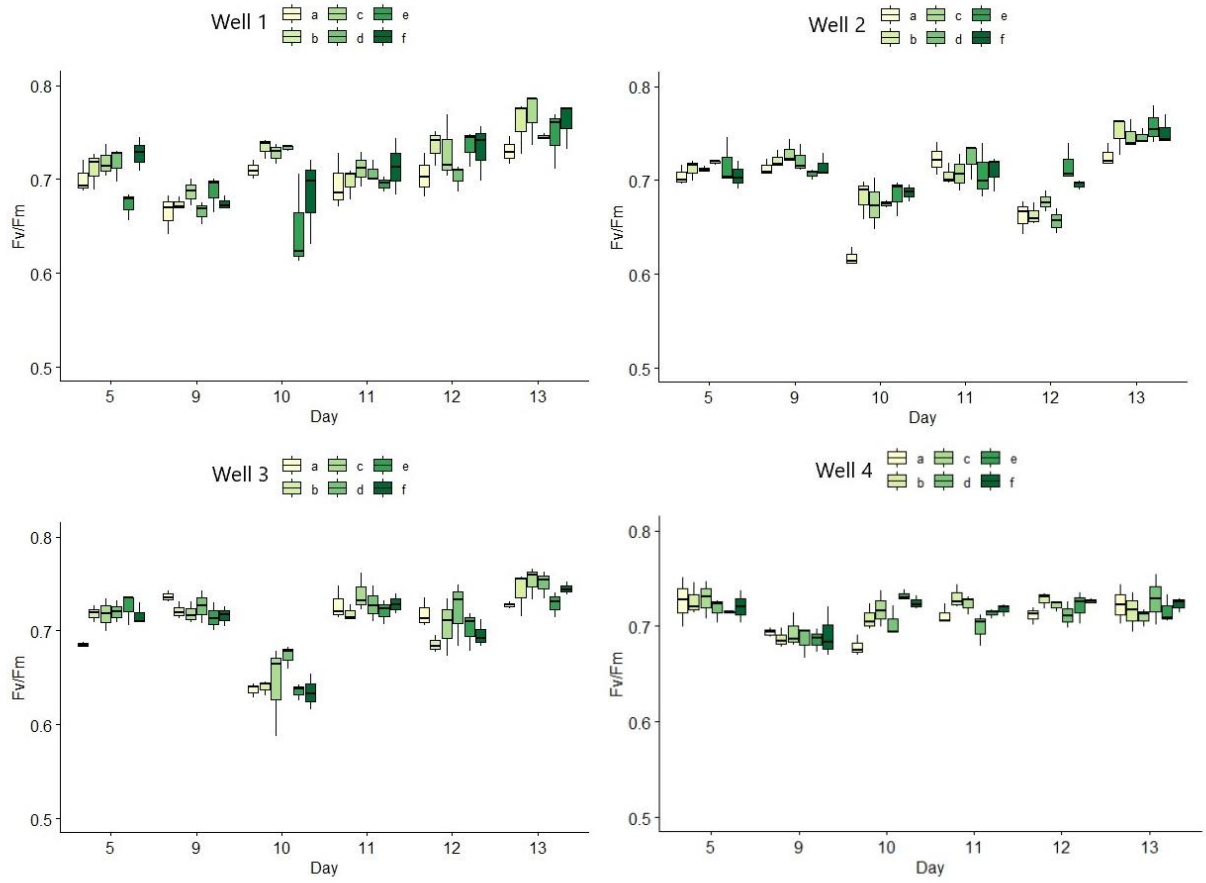

Figure 3.8 Photosynthetic activity (*Fv/Fm*) of all individuals by well plates ("well") and individuals of the plates (named by letters) over duration (Day).

Table 1 Reproduction dates in the well plates ("Well") and their individuals named by letters.

	Well 1	Well 2	Well 3	Well 4
a	13.12.2021	13.12.2021	15.12.2021	17.12.2021
b	13.12.2021	13.12.2021	16.12.2021	16.12.2021
c	14.12.2021	14.12.2021	15.12.2021	16.12.2021
d	13.12.2021	14.12.2021	16.12.2021	16.12.2021
e	14.12.2021	13.12.2021	15.12.2021	15.12.2021
f	13.12.2021	13.12.2021	15.12.2021	15.12.2021

Discussion

Salinity experiment

The experiment showed that the growth of the algae differed depending on the treatment and the days for all species. The highest growth was achieved by the species Ulva californica, followed by *Ulva linza* and *Ulva lacinulata* (lowest). It seems that the species Ulva californica and *U. linza* cope better with lower salinities than *Ulva lacinulata*. The blades of *U. lacinulata* started to fragment at treats PSU 10 and 15 and became pale towards the end.

Adaptation to different salinities differs among *Ulva* species in a wide range. There also might be a relation between different *Ulva* types and salinity tolerance. Thallus-like types such as *Ulva lacinulata* are more able to cope with hypersaline salinities, while tubular types such as *Ulva califiornica* or *U. linza* seem to cope better with lower salinities (Rybak, A. S. ,2018). This fits with the results of the experiment.

Finally, the growth was negatively affected in all species in the low salinities (PSU 20 and below). For the species *Ulva californica*, the treatments of the control groups (PSU 33) and PSU 30 seemed to be the most suitable to achieve maximum growth. For *Ulva linza* this was PSU 30 and PSU 33 (control groups). *Ulva lacinulata* had also achieved the highest growth at 30 PSU. The results are consistent with the results of further literature where most *Ulva* species could achieve their growth optimum around PSU 30 (Chen, B. & Zou, D. ,2015),(Bews, E. et al, 2021). Further experiments in the PSU range of 25 and 35 could be used to provide more accurate information on the salinity optimum of the individual species between the treatments.

Another important aspect is that despite growth, the relative growth rate as well as the maximum PS II photochemical efficiency decreased towards the end in all treatments. This happened evenly over the days and there was no significant difference between the groups in salinity. In the higher salinities (PSU 30) all algae showed minimally better growth rates and photosynthetic activities than in lower salinities but not significantly. Since the decrease in *Fv/Fm* did not occur in the previous experiment with larger tank volumes (*Fv/Fm* remained constant between all days and was less different between treatments), it could be assumed that the smaller volume resulted in a nutrient deficit. Experiment 3, which was run under the

same optimization but had daily water changes, also showed no decrease in photosynthetic activity at PSU 33 during the course of the experiment. Due to the smaller water volumes and insufficient nutrients in the well plates, growth may have slowed down, resulting in minimal damage to the photosystem II. Productivity and photosynthesis can be negatively affected when PSII activity is inhibited by insufficient nutrients (Murchie, E. & Lawson, T, 2013). A study on the effect of nutrients and salinity treatments in *Ulva lactuca* showed that photosynthetic activity decreased significantly in lower nutrient levels (Bews, E. et al, 2021). Interestingly, the lack of nutrients negatively affected *Ulva* metabolism much higher than lower salinity conditions (not significant). It is suggested that *Ulva* could survive and grow well on algae farms under low salinity conditions but with elevated nutrients especially NH4+. Further studies with different nutrient levels and salinity would be interesting.

Efficient photosynthesis in healthy green algae species show *Fv/Fm* values between 0,5 and 0,8 (Higo et al. ,2017). The photosynthetic activity of all species remained mostly within this range in the experiment. On the last day it dropped below 0,5 in some treats, indicating stress of the photosystem II in the second experiment (probably nutrient-related). In the previous experiment, the values ranged from 0,5 (lower salinities) to 0,7 (higher).

The differences of *Fv/Fm* values in the second experiment were not significant in all species, and similar between treats. In the lower salinity treats, with equal *Fv/Fm* between treats but different growth, allocation of energy use may have occurred. Algae from lower salinities may have invested the energy more in the formation of antioxidants, enzymes such as superoxide dismutase, glutathione, starch storage , ß-carotene, ascorbate, to cope better with the lower salinity (Luo, M. B. & Liu, F. ,2011). The products of photosynthesis may also not be efficient to be utilized in growth of germlings and the consumed products might be used in synthesizing some compounds in order balance the intra/extra-cellular osmotic pressure (Chen, B. & Zou, D. ,2015). Furthermore, the question arises whether different stages of development are also influenced by salinity. The germlings can react more sensitively to fluctuations than adult individuals that are adapted to lower salinities.

Life cycle experiment

Temperature remains to be an important factor to induce reproduction in temperate species of *Ulva* beside lunar periodicity, dehydration or periodic increase of light exposition (Balar, N. B. & Mantri, V. A. ,2019). However, the dry shock turned out to be more advantageous to cause larger reproduction events (two days later) than cold and heat shock, which led to a too slow reproduction with few swarmers, rather unsuitable for identification. A too low density of them was very time-consuming in identification, because finding swimming individuals was rare and depended on good timing.

Furthermore, the Table 1 in comparison to Figure 3.8 shows that photosynthetic activity rose and fell on the days of reproduction in a very minimal way. This was relatively balanced (Summary Table 9). So it is not possible to say whether the reproduction process or life cycle stage can be watched out by photosynthetic activity. There are no apparent patterns between the measurements and the reproduction and in some individuals, e.g. well 2 (heat-shock), there are no major changes. Also, it was not possible to prove that the changes in photosynthetic activity during the reproduction are subject to more factors like daytime.

Furthermore, the individuals were apparently clones. All swarmers showed two flagella and therefore, a differentiation of photosynthetic activity between gametes and spores was not possible with this experiment.
However, this experiment can serve as a pre-experiment to further experiments. Different species would have to be used and it is recommended to use the dry shock as reproduction induction. The best time to observe reproduction was between 10 and 12 am should also be used for observation. The use of well plates was also suitable as a quick way of taking measurements with the Imaging PAM. A regular water exchange is necessary to avoid nutrient deficiency and also to remove reproduction inhibitors (Vesty, E. F., Kessler, R. W., Wichard, T. & Coates, J. C. ,2015) or swarming inhibitors which prevents the release of swarmers (Wichard, T. & Oertel, W. ,2010). Because the inhibitors are not specific between species it is expected, that also our used *Ulva* strains would synthesize them. Vesty et al. could show a induction of gamet reproduction by cutting *Ulva linza* into small pieces. This also might be tested. They also draw attention to the fact that cultivation under sterile laboratory conditions is proving difficult. For example, it has been shown for several species of green algae that the epiphytic

bacterial populations with which they are naturally associated are absolutely necessary for proper development and subsequent morphogenesis.

Spoerner et al. define a number of bacterial species and partially purified substances that are required for proper morphogenesis of *Ulva* (Spoerner et al., 2012). In the experiments of Vesty et al., it was speculated whether the specific bacterial strains might not have resulted in less inhibitors due to digestion. Since this plays a role in cultivation, further experiments should use cultures that take this into account. Since *Ulva californica* was cultivated for years at AWI, the biome may have been affected, influencing the life cycle.

In order to be able to make a real statement about whether the Imaging PAM is suitable for distinguishing between gameto-and sporophytes, reliable comparisons between species and different life cycle stages should be investigated.

Conclusion

The study solved some questions but also raised new ones that can be addressed for further scaling and optimization of the biomass harvesting process. It was shown how growth and photosynthetic activity were affected by salinity and that for all species PSU 30 seemed to be most suitable. The hypothesis that the growth and photosynthetic activity differs between the salinity treatments could be confirmed. Even though the *Fv/Fm* values decreased in the end probably due to nutrients and damage to photosystem II in all of them, they were not significant different between the treatments, indicating allocation of energy in the background of different growth. Further studies in the PSU range between 25 and 35 or studies between salinity and nutrients can provide information for qualitative biomass production. Attention should be paid to nutrient supply in the experiments when smaller water volumes are used like here after the optimization.

After the third experiment it was possible to show what should be taken into account in further investigations for the differentiation of gameto-and sporophytes by using PAM fluometry. Whether the imaging PAM method is really suitable for the differentiation of different life cycle stages has to be investigated with other species and further experiments and could not be answered with this work. The hypothesis that the photosynthetic activity varies on reproductive days and that there is a difference between the gameto-and sporophytes measurable could not be confirmed. It wasn't possible to compare different life cycle stages, because the germlings are probably clones.

However, the dry shock method proved to be the best variant of induced reproduction. Observations and identification should take place at best between 10 and 12 am and identification of swarmers using the Lugol's solution was great for counting the flagella. The experimental setting lends itself well to further experiments with other species. PAM microscopy could be an interesting and more precise alternative. Or a completely different way like proteindifferencing between gameto-and sporophytes (Hoxmark, R. C. ,1976).

References

10 Erschreckende Fakten über Plastik. (2021, 27. März). National Geographic. Abgerufen am 26. Januar 2022, von https://www.nationalgeographic.de/10-erschreckende-fakten-uber-plastik

Alfred-Wegener-Institut (2021). Mak-Pak Scale-Up. Zugriff am 27.09.2021. Verfügbar unter: https://www.awi.de/forschung/besondere-gruppen/aquakultur/marine-aquakultur/forschung/mak-pak-1.html

Arioli, T., Mattner, S. W. & Winberg, P. C. (2015). Applications of seaweed extracts in Australian agriculture: past, present and future. *Journal of Applied Phycology*, *27*(5), 2007–2015. https://doi.org/10.1007/s10811-015-0574-9

Balar, N. B. & Mantri, V. A. (2019). Insights into life cycle patterns, spore formation, induction of reproduction, biochemical and molecular aspects of sporulation in green algal genus Ulva: implications for commercial cultivation. *Journal of Applied Phycology*, *32*(1), 473–484. https://doi.org/10.1007/s10811-019-01959-7

Battacharyya, D., Babgohari, M. Z., Rathor, P. & Prithiviraj, B. (2015). Seaweed extracts as biostimulants in horticulture. *Scientia Horticulturae*, *196*, 39–48. https://doi.org/10.1016/j.scienta.2015.09.012

Bews, E., Booher, L., Polizzi, T., Long, C., Kim, J. H. & Edwards, M. S. (2021). Effects of salinity and nutrients on metabolism and growth of Ulva lactuca: Implications for bioremediation of coastal watersheds. *Marine Pollution Bulletin*, *166*, 112199. https://doi.org/10.1016/j.marpolbul.2021.112199

Bouga, M. & Combet, E. (2015). Emergence of Seaweed and Seaweed-Containing Foods in the UK: Focus on Labeling, Iodine Content, Toxicity and Nutrition. *Foods*, *4*(4), 240–253. https://doi.org/10.3390/foods4020240

B.U.N.D. (2019, Juli). *Plastikatlas 2019 – Daten und Fakten über eine Welt voller Kunststoff* (2. Auflage Aufl.).

Chen, B. & Zou, D. (2015). Altered seawater salinity levels affected growth and photosynthesis of Ulva fasciata (Ulvales, Chlorophyta) germlings. *Acta Oceanologica Sinica*, *34*(8), 108–113. https://doi.org/10.1007/s13131-015-0654-3

Dhargalkar, V. K. (2006, 14. November). Seaweed: Promising plant of the millennium. Science and Culture. https://drs.nio.org/drs/handle/2264/489

Dawczynski, C., Schäfer, U., Leiterer, M. & Jahreis, G. (2007, November). Nutritional and Toxicological Importance of Macro, Trace, and Ultra-Trace Elements in Algae Food Products. *Journal of Agricultural and Food Chemistry*. https://pubs.acs.org/action/doSearch?field1=Contrib&text1=Christine++Dawczynski

Ferdouse, F., Holdt, S. L., Smith, R., Murúa, P., & Yang, Z. (2018). The global status of seaweed production, trade and utilization. Globefish Research Programme, 124, I. Zugriff am 28.12.2021. Verfügbar unter: https://issuu.com/globefish/docs/the_global_status_of_seaweed_production__trade_and/8?ff&showOtherPublicationsAsSuggestions=true

Guttman, L., Boxman, S. E., Barkan, R., Neori, A. & Shpigel, M. (2018). Combinations of Ulva and periphyton as biofilters for both ammonia and nitrate in mariculture fishpond effluents. *Algal Research*, *34*, 235–243. https://doi.org/10.1016/j.algal.2018.08.002

Higo, S., Maung-Saw-Htoo-Thaw, Yamatogi, T., Ishida, N., Hirae, S. & Koike, K. (2017). Application of a pulse-amplitude-modulation (PAM) fluorometer reveals its usefulness and robustness in the prediction of Karenia mikimotoi blooms: A case study in Sasebo Bay, Nagasaki, Japan. *Harmful Algae*, *61*, 63–70. https://doi.org/10.1016/j.hal.2016.11.013

Hochschule Bremerhaven (2021). Mak-Pak / Mak-Pak Scale Up. Zugriff am 27.09.2021. Verfügbar unter: https://www.hs-bremerhaven.de/forschung/forschungsprojekte/mak-pak-mak-pak-scale-up/

Hoxmark, R. C. (1976). Protein composition of different stages in the life cycle of Ulva mutabilis, Fyn. *Planta*, *130*(3), 327–332. https://doi.org/10.1007/bf00387841

Lawson, T. (2013, 3. August). Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. In E. H. Murchie (Hrsg.), *Journal of Experimental Botany Volume 64* (S. 3983–3998). E.H. Murchie, T. Lawson. https://doi.org/10.1093/jxb/ert208

Luo, M. B. & Liu, F. (2011). Salinity-induced oxidative stress and regulation of antioxidant defense system in the marine macroalga Ulva prolifera. *Journal of Experimental Marine Biology and Ecology*, *409*(1–2), 223–228. https://doi.org/10.1016/j.jembe.2011.08.023

Miller, L. (2011, 12. August). *Measuring leaf or algae blade area in a digital photo with ImageJ*. https://lukemiller.org. https://lukemiller.org/index.php/2011/08/measuring-leaf-or-algae-blade-area-in-a-digital-photo-with-imagej/

Murchie, E. & Lawson, T. (2013). Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *Journal of Experimental Botany*, *64*(13), 3983–3998. https://doi.org/10.1093/jxb/ert208

P. (2016, 13. September). *Zahlen und Fakten zu Plastiktüten*. h_da | fbg ökologie. Abgerufen am 26. Januar 2022, von https://designoekologie.com/2016/05/30/7504/

Potter, E. E., Thornber, C. S., Swanson, J. D. & McFarland, M. (2016). Ploidy Distribution of the Harmful Bloom Forming Macroalgae Ulva spp. in Narragansett Bay, Rhode Island, USA, Using Flow Cytometry Methods. *PLOS ONE*, *11*(2), e0149182. https://doi.org/10.1371/journal.pone.0149182

Rasband, W.: ImageJ:, URL http://imagej.nih.gov/ij, 2021

Rybak, A. S. (2018). Species of Ulva (Ulvophyceae, Chlorophyta) as indicators of salinity. *Ecological Indicators*, *85*, 253–261. https://doi.org/10.1016/j.ecolind.2017.10.061

Scherner, F., Ventura, R., Barufi, J. B. & Horta, P. A. (2013a). Salinity critical threshold values for photosynthesis of two cosmopolitan seaweed species: Providing baselines for potential shifts on seaweed assemblages. *Marine Environmental Research*, *91*, 14–25. https://doi.org/10.1016/j.marenvres.2012.05.007

Scherner, F., Ventura, R., Barufi, J. B. & Horta, P. A. (2013b). Salinity critical threshold values for photosynthesis of two cosmopolitan seaweed species: Providing baselines for potential shifts on seaweed assemblages. *Marine Environmental Research*, *91*, 14–25. https://doi.org/10.1016/j.marenvres.2012.05.007

Schymanski, D., Goldbeck, C., Humpf, H. U. & Fürst, P. (2018). Analysis of microplastics in water by micro-Raman spectroscopy: Release of plastic particles from different packaging

into mineral water. *Water Research*, *129*, 154–162. https://doi.org/10.1016/j.watres.2017.11.011

Spoerner, M., Wichard, T., Bachhuber, T., Stratmann, J. & Oertel, W. (2012). Growth and Thallus Morphogenesis ofUlva mutabilis(Chlorophyta) Depends on A Combination of Two Bacterial Species Excreting Regulatory Factors. *Journal of Phycology*, *48*(6), 1433–1447. https://doi.org/10.1111/j.1529-8817.2012.01231.x

Vesty, E. F., Kessler, R. W., Wichard, T. & Coates, J. C. (2015). Regulation of gametogenesis and zoosporogenesis in Ulva linza (Chlorophyta): comparison with Ulva mutabilis and potential for laboratory culture. *Frontiers in Plant Science*, *6*. https://doi.org/10.3389/fpls.2015.00015

Wichard, T. & Oertel, W. (2010). GAMETOGENESIS AND GAMETE RELEASE OFULVA MUTABILISANDULVA LACTUCA(CHLOROPHYTA): REGULATORY EFFECTS AND CHEMICAL CHARACTERIZATION OF THE “SWARMING INHIBITOR”. *Journal of Phycology*, *46*(2), 248–259. https://doi.org/10.1111/j.1529-8817.2010.00816.x

Appendix

Salinity Experiment

A-1 Summary Table: Mean values, standard deviation (sd) and standard error (se) of the total surface area (TSA) in cm^2 for *Ulva linza* grouped by day and salinity treatment in PSU.

Day	Salinity	TSA_{mean}	sd	se
0	10	0,070	0,029	0,017
0	15	0,129	0,096	0,055
0	20	0,115	0,023	0,013
0	30	0,215	0,140	0,081
3	10	0,203	0,064	0,037
3	15	0,380	0,297	0,171
3	20	0,360	0,092	0,053
3	30	0,645	0,433	0,250
7	10	0,877	0,242	0,140
7	15	1,218	0,384	0,222
7	20	1,350	0,281	0,162
7	30	1,642	0,484	0,279
14	10	1,047	0,300	0,173
14	15	1,647	0,506	0,292
14	20	1,991	0,249	0,144
14	30	2,357	0,275	0,159
17	10	1,292	0,255	0,147
17	15	1,896	0,397	0,229
17	20	2,308	0,241	0,139
17	30	2,639	0,251	0,145
21	10	1,477	0,237	0,137
21	15	2,136	0,424	0,245
21	20	2,647	0,330	0,191
21	30	3,049	0,320	0,185

A-2Summary Table: Mean values, standard deviation (sd) and standard error (se) of the total surface (TSA) in cm^2 for *Ulva californica* grouped by day and salinity treatment in PSU.

Day	Salinity	TSA_{mean}	sd	se
0	10	0,335	0,223	0,129
0	15	0,282	0,072	0,042
0	20	0,354	0,146	0,084
0	30	0,134	0,028	0,016
3	10	0,471	0,089	0,051
3	15	0,202	0,041	0,024
3	20	0,344	0,242	0,140
3	30	0,365	0,168	0,097
7	10	0,947	0,213	0,123
7	15	0,712	0,130	0,075
7	20	0,772	0,150	0,086
7	30	0,858	0,227	0,131
14	10	1,199	0,334	0,193
14	15	0,909	0,031	0,018
14	20	0,949	0,169	0,098
14	30	1,244	0,139	0,080
17	10	1,469	0,230	0,133
17	15	1,141	0,070	0,040
17	20	1,182	0,299	0,173
17	30	1,661	0,151	0,087
21	10	1,704	0,270	0,156
21	15	1,551	0,072	0,042
21	20	1,408	0,242	0,140
21	30	2,110	0,387	0,224

A-3 Summary Table: Mean values, standard deviation (sd) and standard error (se) of the total surface area (TSA) in cm^2 for *Ulva lacinulata* grouped by day and salinity treatment in PSU.

Day	Salinity	TSA_{mean}	sd	se
0	10	0,038	0,010	0,006
0	15	0,089	0,005	0,003
0	20	0,106	0,006	0,004
0	30	0,097	0,045	0,026
3	10	0,051	0,005	0,003
3	15	0,120	0,042	0,024
3	20	0,212	0,085	0,049
3	30	0,212	0,042	0,024
7	10	0,046	0,011	0,006
7	15	0,310	0,078	0,045
7	20	0,604	0,320	0,185
7	30	0,940	0,158	0,091
14	10	0,049	0,014	0,008
14	15	0,423	0,153	0,088
14	20	0,701	0,302	0,174
14	30	1,314	0,020	0,011
17	10	0,132	0,063	0,036
17	15	0,516	0,167	0,096
17	20	0,783	0,305	0,176
17	30	1,639	0,293	0,169
21	10	0,099	0,017	0,010
21	15	0,485	0,137	0,079
21	20	0,779	0,204	0,118
21	30	1,657	0,321	0,185

A-4 Summary Table: Mean values, standard deviation (sd) and standard error (se) of maximum efficiency of PSII photochemistry (F_v/F_m) for *Ulva linza* grouped by day and salinity treatment in PSU..

Day	Treat	Fv/Fm	sd	se
0	10	0,47858265	0,01247528	0,00720261
0	15	0,64777685	0,07092762	0,04095008
0	20	0,39685816	0,098138	0,05666
0	30	0,61700175	0,04210433	0,02430895
3	10	0,71584207	0,01208687	0,00697836
3	15	0,71392685	0,01374698	0,00793682
3	20	0,70690329	0,01323703	0,0076424
3	30	0,70525144	0,01162438	0,00671134
7	10	0,68695398	0,02883348	0,01664702
7	15	0,68201136	0,05543894	0,03200769
7	20	0,66314328	0,04366067	0,0252075
7	30	0,66230117	0,05088913	0,02938085
14	10	0,46500662	0,21126242	0,12197241
14	15	0,58252342	0,09977334	0,05760416
14	20	0,59417954	0,03585348	0,02070001
14	30	0,58539545	0,03853803	0,02224994
17	10	0,6217783	0,01927907	0,01113078
17	15	0,63943684	0,02284104	0,01318728
17	20	0,53115723	0,07238254	0,04179008
17	30	0,57211727	0,03809294	0,02199297
21	10	0,5372454	0,01333002	0,00769609
21	15	0,55523308	0,00960934	0,00554796
21	20	0,47556432	0,05201089	0,0300285
21	30	0,51544676	0,07377852	0,04259605

A-5 Summary Table: Mean values, standard deviation (sd) and standard error (se) of maximum efficiency of PSII photochemistry (F_v/F_m) for *Ulva linza* grouped by day and salinity treatment in PSU.

Day	Treat	Fv/Fm	sd	se
0	10	0,35601784	0,03973946	0,02294359
0	15	0,30108457	0,03169134	0,01829701
0	20	0,40960826	0,08748302	0,05050835
0	30	0,28589917	0,02686328	0,01550952
3	10	0,67035844	0,04560875	0,02633222
3	15	0,67475332	0,0357255	0,02062613
3	20	0,6843941	0,03172944	0,018319
3	30	0,68915096	0,02324862	0,0134226
7	10	0,5532865	0,05850715	0,03377912
7	15	0,64080924	0,06647761	0,03838087
7	20	0,62402581	0,07305484	0,04217823
7	30	0,64569893	0,05534942	0,031956
14	10	0,52759525	0,04146736	0,02394119
14	15	0,53304902	0,04786976	0,02763762
14	20	0,5371522	0,03030751	0,01749805
14	30	0,55041928	0,05439	0,03140208
17	10	0,60465174	0,04666553	0,02694235
17	15	0,54912473	0,0371248	0,02143401
17	20	0,50889947	0,02187896	0,01263182
17	30	0,63445703	0,03913977	0,02259736
21	10	0,49289604	0,04597535	0,02654388

A-6 Summary Table: Mean values, standard deviation (sd) and standard error (se) of maximum efficiency of PSII photochemistry (F_v/F_m) for *Ulva linza* grouped by day and salinity treatment in PSU..

Day	Treat	Fv/Fm	sd	se
0	10	0,48200764	0,08061033	0,0465404
0	15	0,53670553	0,06972486	0,04025567
0	20	0,54885202	0,09006904	0,05200138
0	30	0,19142938	0,10704472	0,0618023
3	10	0,573074	0,06140886	0,03545442
3	15	0,5708421	0,09292452	0,05365
3	20	0,52091111	0,09039223	0,05218798
3	30	0,56212297	0,09013219	0,05203784
7	10	0,63870959	0,02929	0,01691059
7	15	0,69160967	0,00782518	0,00451787
7	20	0,67199387	0,01464238	0,00845378
7	30	0,68868452	0,00666731	0,00384937
14	10	0,5574048	0,03843748	0,02219189
14	15	0,50407185	0,06596903	0,03808724
14	20	0,41922585	0,19548397	0,11286272
14	30	0,47850175	0,01654536	0,00955247
17	10	0,55837887	0,0712449	0,04113326
17	15	0,59614213	0,05390488	0,031122
17	20	0,47461437	0,15085478	0,08709605
17	30	0,54104364	0,04037687	0,0233116
21	10	0,5255869	0,0136968	0,00790785

A-7 Summary Table: maximum efficiency of PSII photochemistry (F_v/F_m) for *Ulva californica* on reproductive days and Well 1 and 2, which was treated by coldshock reproduction induction.

Day	Well 1	FvFm	sd	se	Well 2	FvFm	sd	se
3	1a	0,701	0,014	0,008	2a	0,705	0,008	0,005
3	1b	0,711	0,016	0,009	2b	0,712	0,009	0,005
3	1c	0,718	0,014	0,008	2c	0,712	0,002	0,001
3	1d	0,718	0,015	0,009	2d	0,719	0,002	0,001
3	1e	0,673	0,012	0,007	2e	0,717	0,020	0,012
3	1f	0,727	0,014	0,008	2f	0,705	0,012	0,007
8	1a	0,665	0,017	0,010	2a	0,713	0,007	0,004
8	1b	0,674	0,005	0,003	2b	0,722	0,007	0,004
8	1c	0,687	0,012	0,007	2c	0,729	0,011	0,006
8	1d	0,665	0,010	0,006	2d	0,721	0,012	0,007
8	1e	0,687	0,016	0,009	3e	0,707	0,005	0,003
8	1f	0,675	0,006	0,004	2f	0,715	0,010	0,006
9	1a	0,710	0,008	0,005	2a	0,618	0,008	0,005
9	1b	0,734	0,009	0,005	2b	0,682	0,017	0,010
9	1c	0,728	0,009	0,005	2c	0,674	0,022	0,013
9	1d	0,733	0,003	0,002	2d	0,675	0,003	0,002
9	1e	0,447	0,202	0,116	4e	0,684	0,016	0,009
9	1f	0,683	0,038	0,022	2f	0,687	0,008	0,004
11	1a	0,695	0,024	0,014	2a	0,723	0,014	0,008
11	1b	0,697	0,014	0,008	2b	0,705	0,009	0,005
11	1c	0,711	0,015	0,009	2c	0,708	0,016	0,009
11	1d	0,707	0,010	0,006	2d	0,723	0,016	0,009
11	1e	0,695	0,007	0,004	3e	0,707	0,024	0,014
11	1f	0,713	0,025	0,014	2f	0,710	0,016	0,009
11	1a	0,704	0,019	0,011	2a	0,662	0,015	0,008
12	1b	0,736	0,016	0,009	2b	0,663	0,010	0,006
12	1c	0,730	0,029	0,017	2c	0,677	0,009	0,005
12	1d	0,703	0,012	0,007	2d	0,657	0,011	0,006
12	1e	0,735	0,016	0,009	4e	0,716	0,016	0,009
12	1f	0,732	0,025	0,014	2f	0,695	0,004	0,002
13	1a	0,731	0,012	0,007	2a	0,726	0,010	0,006
13	1b	0,760	0,023	0,014	2b	0,751	0,017	0,010
13	1c	0,770	0,023	0,014	2c	0,747	0,013	0,007
13	1d	0,746	0,002	0,001	2d	0,746	0,007	0,004
13	1e	0,747	0,026	0,015	5e	0,758	0,016	0,009
13	1f	0,762	0,020	0,012	2f	0,752	0,013	0,007

A-8 Summary Table: maximum efficiency of PSII photochemistry (F_v/F_m) for *Ulva californica* on reproductive days and Well 3 and 4, which was treated by heatshock reproduction induction.

Day	Well 3	FvFm	sd	se	Well 4	FvFm	sd	se
3	3a	0,685	0,002	0,001	4a	0,726	0,021	0,012
3	3b	0,718	0,007	0,004	4b	0,727	0,014	0,008
3	3c	0,718	0,014	0,008	4c	0,729	0,016	0,009
3	3d	0,721	0,009	0,005	4d	0,718	0,010	0,006
3	3e	0,726	0,014	0,008	4e	0,715	0,001	0,001
3	3f	0,716	0,009	0,005	4f	0,721	0,014	0,008
8	3a	0,736	0,005	0,003	4a	0,694	0,004	0,002
8	3b	0,721	0,007	0,004	4b	0,687	0,008	0,005
8	3c	0,719	0,009	0,005	4c	0,694	0,015	0,009
8	3d	0,726	0,014	0,008	4d	0,686	0,014	0,008
8	4e	0,715	0,012	0,007	5e	0,686	0,010	0,006
8	3f	0,716	0,009	0,005	4f	0,691	0,022	0,012
9	3a	0,637	0,006	0,004	4a	0,679	0,009	0,005
9	3b	0,640	0,007	0,004	4b	0,708	0,011	0,006
9	3c	0,644	0,040	0,023	4c	0,718	0,016	0,009
9	3d	0,674	0,010	0,006	4d	0,703	0,013	0,008
9	5e	0,635	0,007	0,004	6e	0,732	0,005	0,003
9	3f	0,634	0,016	0,009	4f	0,724	0,006	0,003
11	3a	0,728	0,015	0,008	4a	0,711	0,008	0,005
11	3b	0,718	0,007	0,004	4b	0,730	0,010	0,006
11	3c	0,739	0,016	0,010	4c	0,724	0,008	0,005
11	3d	0,729	0,015	0,009	4d	0,699	0,014	0,008
11	6e	0,721	0,011	0,006	7e	0,714	0,004	0,002
11	3f	0,729	0,009	0,005	4f	0,718	0,006	0,003
11	3a	0,718	0,013	0,007	4a	0,712	0,007	0,004
12	3b	0,685	0,008	0,004	4b	0,728	0,007	0,004
12	3c	0,706	0,025	0,014	4c	0,722	0,005	0,003
12	3d	0,722	0,028	0,016	4d	0,712	0,011	0,006
12	7e	0,702	0,017	0,010	8e	0,721	0,013	0,008
12	3f	0,696	0,012	0,007	4f	0,727	0,002	0,001
13	3a	0,728	0,003	0,002	4a	0,723	0,017	0,010
13	3b	0,743	0,019	0,011	4b	0,716	0,017	0,010
13	3c	0,753	0,014	0,008	4c	0,710	0,008	0,005
13	3d	0,750	0,012	0,007	4d	0,728	0,021	0,012
13	8e	0,729	0,011	0,006	9e	0,716	0,012	0,007
13	3f	0,745	0,006	0,003	4f	0,723	0,006	0,004

A-9 Summary Table: reproductionobervations of Ulva californica in the third experiment. Blue colors represent decreases of photosynthetic activity, lightgreen increase. The ratio off green and blue is balanced.

	13.12.	14.12.	15.12.	16.12.	17.12.
1a	x				
1b	x				
1c		x			
1d	x				
1e		x			
1f	x				
2a	x				
2b	x				
2c		x			
2d		x			
2e	x				
2f	x				
3a			x		
3b				x	
3c			x		
3d				x	
3e			x		
3f			x		
4a					x
4b				x	
4c				x	
4d				x	
4e			x		
4f			x		

Acknowlegements

I would like to thank my supervisor, Dr. Laurie C. Hofmann, and the Marine Aquaculture Section for allowing me to participate in such an innovative, sustainable, and exciting project and the ability for the tremendous amount of learning. This means a lot to me!

I would also like to thank Dr. Inka Bartsch and the Rocky Shore Ecology Section for allowing me to conduct my experiments in their labs at AWI.

I thank Isabel Cardoso for her support and help and would also like to express special thanks to my family and friends.

Eidesstattliche Erklärung

Ich erkläre, dass ich die vorgelegte Arbeit selbständig angefertigt, dabei keine anderen Hilfsmittel als die im Quellen- und Literaturverzeichnis genannten benutzt, alle aus Quellen und Literatur, einschließlich des Internets, wörtlich oder sinngemäß entnommenen Stellen als solche kenntlich gemacht und auch die Fundstellen einzeln nachgewiesen habe.

Diese Arbeit ist in gleicher oder ähnlicher Form noch bei keiner anderen Prüfungsbehörde eingereicht worden.

Musterstadt, Datum Handschriftliche Unterschrift