Master's thesis

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Department of Biology

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Illustrating the Potential of Ulva sp.:

Development of an Explorative Model for the Multiculture of *Ulva sp.* and *Littorina littorea* in a Recirculating Aquaculture System (RAS) Waste Stream

Master's thesis in Ocean Resources
Supervisor: Kjell Inge Reitan
Co-supervisor: Silje Forbord
May 2022

NTNU
Kunnskap for en bedre verden

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May 2022, Trondheim

Mila D. Vlottes

Preface

This thesis has been completed at the Department of Biology at NTNU in collaboration with SINTEF Ocean. This study was a part of the project *'Oppdrett av Vanlig Strandsnegl'* funded by RFF Trøndelag (#299075), Nofitech and Statsnail AS, and coordinated by SINTEF Ocean.

The experiments were carried out within the framework of the research infrastructure Norwegian Center for Plankton Technology (245937/F50) hosted by SINTEF Ocean and NTNU.

Abstract

The cultivation of seaweed species in fishpond effluent, characterized by high ammonium concentrations, has shown to successful and often high removal efficiencies can be attained. However, the high nitrate concentration from RAS wastewater could impact this potential due to the additional energy seaweed requires to take up this nutrient. To explore the effects of RAS wastewater on *Ulva sp.*, this thesis examined the cultivation potential, uptake and removal efficiency. To illustrate how the additionally cultivated *Ulva sp.* biomass could be utilized, an explorative model has been developed that examined the co-culture of *Ulva sp.* and *Littorina littorea* in RAS wastewater.

This study demonstrated that *Ulva sp.* cultivated in the wastewater of a RAS facility, showed enhanced growth and chemical composition, compared to *Ulva sp.* cultivated in artificial seawater. On average, the specific growth rate and protein value were 337 and 319 % higher, respectively. Additionally, both the C:N and N:P ratios in the tissue showed to be around optimal levels of approximately 1:9 and 1:28, respectively. This study found that varying concentrations between 100-25% RAS wastewater in the cultivation medium did not significantly affect these results. However, trends in the growth and chemical composition displayed that 25% RAS wastewater concentration produced the largest growth over time and the highest protein contents compared to 100% RAS water concentration. The uptake experiments showed that *Ulva sp.* can successfully take up nitrate and could attain removal efficiencies between 55- 80%, with a specific uptake rate between 5600 and 2500 µg NO_3^- g DW^{-1} day^{-1}, respectively. This specific uptake rate and removal efficiency, in comparison to the ammonium, were 330 and 2 times higher, respectively. The high nutrient uptake of NO_3^- showed that the bioremediation potential of *Ulva sp.* is not limited to ammonium rich water.
To demonstrate how the additional biomass could be used, an explorative co-culture with *Ulva sp. and L. littorea* in RAS wastewater was simulated. Over a five-year period, using a biomass of 1344 kg FW *Ulva sp.* cultivated in RAS wastewater, more than 300 000 snails could be harvested. The explorative model showed that the snails can continuously graze on the seaweed biomass and that over a five-year period could be harvested at a steady rate. The robustness scenarios showed that reducing both mortality of *Ulva sp.* biomass an*d L. littorea* offered most opportunities to increase output, whilst maintaining the same input.

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List of abbreviations

Table a. List of abbreviations

ABBREVIATION	MEANING
ANOVA	Analysis of Variance
AS	Artificial seawater
C	Carbon
CO_2	Carbon Dioxide
C:N	Carbon: nitrogen
D	Days
DW	Dry weight
Eq.	Equation
FW	Fresh weight
IMTA	Integrated Multi-trophic aquaculture
Ind.	Individual
N	Nitrogen
NH_3	Ammonia
Nh_4^+	Ammonium
NO_2^-	Nitrite
NO_3^-	Nitrate
N:P	Nitrogen: phosphorous
NTNU	Norwegian University of Science and Technology
P	Phosphorous
PO_4^{3-}	Phosphate
OP	Organic phosphates
RAS	Recycling aquaculture system
RE	Removal efficiency
SINTEf	Stiftelsen for industriell og teknisk forskning
SGR	Specific growth rate
TAN	Total ammonium nitrogen
TN	Total nitrogen
TR	Treatment
USD	United States Dollar
V	Specific uptake rate

1. Introduction

1.1 Growth and Challenges of the Norwegian Aquaculture sector

In 2018, the Norwegian marine food sector was valued at 10 815 million USD, with a production of 4 million tons of marine organisms from both fisheries and aquaculture production (OECD, 2021). Of this, 75 % of the revenue was generated by aquaculture, highlighting the importance of this sector (OECD, 2021) and its leading position in the country's marine food industry (Thorvalsden et al., 2018).

Both the industry and the Norwegian government share a vision to achieve a five-fold increase in production by 2050 (Krøvel et al., 2019; Sandersen & Kvalvik, 2015). However, to secure constant growth and reach its set goal, it is important that harmful environmental effects associated with aquaculture are given sufficient attention. These environmental effects have been increasingly the issue of public concern and media discussions have become more negative about the industry (Martins et al., 2010; Van Rijn, 2013; Young et al., 2019). The environmental issues that are raised comprise a wide spectrum and include unsustainable feed ingredients, escaped fish from aquaculture sites and discharge of waste into marine ecosystems (Martins et al., 2010). The nutrient discharge from the Norwegian aquaculture sector can be compared to sewage discharge of around 10 million people (Olaussen, 2018) and may result in nutrient and chemical pollution and alterations in seafloor compositions (Ellingsen et al., 2009; Olaussen, 2018; Rust et al., 2014).

1.2 Aquaculture Waste

The nutrient waste, resulting from feeding and fish metabolism, can be categorized by the different properties, such as inorganic and organic nutrients and the size of the particles. Firstly, the inorganic nutrient waste from aquaculture results from the metabolic and respiration processes and the leakage of nutrients from solid waste. This produces ammonium (NH_4^+), carbon dioxide (CO_2) and orthophosphates (PO_4^{3-}) (Lander et al., 2013; Reid et al., 2013). The main product of inorganic waste is nitrogen in the form of ammonium (Cohen & Neori, 1991; Dauda et al., 2019). Ammonium exists in an un-ionized form and the ionized form (NH_3 and NH_4^+), together forming the total ammonium nitrogen (TAN) (Dauda et al., 2019). Ammonium is considered to be toxic to organisms in the cultivation medium and in the receiving water body (Dauda et al., 2019). The other two important forms of nitrogen waste are nitrite (NO_2^-)

and nitrate (NO_3^-). The former, NO_2^-, is the intermediate step of the oxidation from TAN to nitrate. NO_2^- is considered to be toxic to marine organisms, including fish, which show an active uptake of the molecule (Fjellheim et al., 2016). This can result in a lower ability of blood to transport oxygen (Fjellheim et al., 2016). The latter, NO_3^-, is the end-product of ammonium oxidation and is considered far less toxic then the two other forms of nitrogen. It is, however, still capable of causing eutrophication in the receiving water body (Dauda et al., 2019). In industrial aquaculture systems, only 10-30% of the added nitrogen is retrieved as harvested fish and as much as 50% of nitrogen is excreted in dissolved form. (Zhang et al., 2016).

Secondly, organic waste is produced from feed waste or feces. They can be small suspended and slowly sinking or large particles that are heavier and more directly deposited on the (seafloor) bottom (Reid et al., 2013). Whereas nitrogen is mostly excreted as inorganic nutrients, the majority of the phosphorous is released as particulate material and therefore is an organic nutrient product (Dauda et al., 2019). Phosphorous is not considered toxic to the cultured fish but may cause nutrient pollution (Dauda et al., 2019). Wang et al. (2013) calculated that around 76% of the phosphorous input through feed was lost to the environment.

1.3 Solutions and Opportunities

The release of nutrients offers potential feeding niches for lower trophic levels. By incorporating different trophic levels such as mollusks and/or macroalgae species, recent biological engineering concepts are able to convert mono-aquaculture into multiculture-aquaculture systems. This method, described as Integrated Multitrophic Aquaculture (IMTA) has the potential to increase the overall sustainability of a fish farm (Nobre et al., 2010; Troell et al., 2009). IMTA can be described as a natural recycling approach in which the waste and byproducts from the main species becomes a food source for another species that occupies a lower trophic level (Filgueira et al., 2017). The release of inorganic nutrients, as described before, can be taken up by inorganic extractive species. Dissolved inorganic nitrogen and phosphorous excreted from fish are available for macroalgae and can be rapidly taken up (Wang et al., 2012; Reid et al., 2013). Several seaweed species have the ability to assimilate and accumulate the nutrients into their tissue and offering the service long-term reservoirs (Wang et al., 2014). The organic particles can be consumed by filter feeders and detritus feeders (Reid et al., 2013). Mollusks are commonly used to filter the small particles from the water body and are suitable for IMTA due to their wide environmental distribution and the ability to be cultured in high densities (Filgueira et al., 2017). Detritus feeders, such as sea urchins and

sea cucumbers can consume the particulate waste from aquaculture facilities and have shown to exhibit enhanced growth and survival (Cubillo et al., 2016).

Another solution that is proposed for the nutrient discharges is a shift from open net pens to closed facilities (Ayer & Tyedmers, 2009; Olaussen, 2018). An example of a closed type system is a Recycling Aquaculture System (RAS), where water is recirculated into tanks after passing through a series of mechanical and biological filters (Ayer & Tyedmers, 2009). RAS systems offer waste management, reduced water usage, nutrient recycling, constant water quality and optimal environmental conditions throughout the year, that result in increased welfare and production of the fish (Martins et al., 2010). In RAS, water flows from the central culture tank through filtration tanks, where settleable and suspended solids are removed, ammonium is converted to nitrate and oxygen is again added to the water (Ebeling & Timmons, 2012). The process of removing toxic nitrogen compounds is commonly done by nitrifying bacteria that convert the ammonium into nitrite and then to nitrate (Ebeling & Timmons, 2012). Nitrate is the end-product of the nitrification and can reach elevated levels in RAS of around 75 mg L^{-1} (Fjellheim et al., 2016).

The technique of IMTA can also be successfully applied in RAS systems, where seaweeds can be incorporated and assimilate the excreted nitrogen, phosphate and carbon (Abreu et al., 2011). Whereas the absolute amount of nutrients in RAS produced is similar to marine open net pens, the higher concentration of these nutrients and the smaller volume of these waste streams make them more suitable as a feeding niche for lower trophic levels (Chaitanawisuti et al., 2011). In addition, the link between trophic levels is more straightforward in closed facilities than in marine systems, where the results and successes of IMTA show considerable variation (Cubillo et al., 2016). The extractive species grown in RAS waste stream show to have higher productivity levels due to more constant and higher nutrient availability than species grown in natural medium (Abreu et al., 2011). By incorporating extractive species in RAS, the producer receives an additional viable biomass cultivated in the rest stream of their main product (Chaitanawisuti et al., 2011).

1.4 The Potential of Ulva spp. Cultivation

The genus of *Ulva spp.* has been identified as a suitable candidate for filtering effluents from cultivation tanks (Ben-Ari et al., 2014; Wang et al., 2007). *Ulva spp.* is a genus that belongs to the phylum Chlorophyta, the taxon of green algae (Dominguez & Loret, 2019). It can grow

attached to a surface, sessile or free floating in a body of water and has the ability to reproduce sexually and asexually through fragmentation (Dominguez & Loret, 2019). *Ulva spp.* has a soft sheet like physique that are commonly of two cells thick (Ale et al., 2011; Fortes & Lüning, 1980). This high surface area to volume ratio allows *Ulva sp.* to show high productivity. This, among other qualities, makes *Ulva* spp. is an interesting genus to consider for cultivation (Bews et al., 2021). Additionally, the wide environmental distribution demonstrates the high tolerance of *Ulva spp.* for varying cultivation circumstances (Ben Ari et al., 2014). *Ulva lactuca* cultivated in fishpond effluent has a high growth rate due to its high photosynthetic rates and rapid ability to take up dissolved nitrogen (Ale et al., 2011; Ben-Ari et al., 2014; Nielsen et al., 2012; Shpigel et al., 2018; Toth et al., 2020). Additionally, the high nitrogen flux can result in changes in the chemical composition of the seaweed. Results from Ben-Ari et al. (2014) show that *U. lactuca* cultivated in RAS medium has 2-4 times more protein than regular *U. lactuca*. This high protein value of up to 30% can be compared to a commercial diet for several species (Dominguez & Loret, 2019). As a result, *U. lactuca* can be used as a valuable by-product as for example feed for macroalgivores (Ben-Ari et al., 2014).

However, the success of *Ulva spp.* as a biofilter show variations in the yield, uptake and chemical composition that is dependent on the water composition (Shpigel et al., 2019). RAS waste streams, after the biological and mechanical filter treatments, are characterized by values of NO_3^- that are significantly elevated compared to NH_4^+. The oxidized state of NO_3^- requires more energy to be taken up by the seaweed compared to the reduced state of NH_4^+ (Shpigel et al., 2019). The high presence of NO_3^- specifically in RAS systems may therefore influence the growth and bioremediation potential of this species (Shahar et al., 202). This is supported by Neori (1996) who found that the presence of NO_x limits the performance of *Ulva spp.* as a biofilter. To keep the NO_3^- levels within safe production limits, fresh water needs to be added to the system (Fjellheim et al., 2016). Wastewater from RAS facilities is therefore characterized by high NO_3^- levels. A successful cultivation of *Ulva spp.* in NO_x rich media could result in additional benefits for the RAS producer as the waste product of their facility could be utilized for the cultivation of a low trophic species.

1.5 Low Trophic Multicultures

Repeatedly, research has shown that *Ulva spp.* biomass has been the preferred algae to be grazed by several gastropods, including for *Littorina littorea*. *L. littorea*, more generally known

as the common periwinkle, is a general herbivore and shows preference for green sheet like algae such as *Ulva spp.* (Bakke, 1988; Cummins et al., 2002; Davies & Falconer et al., 2001; Wilhelmsen & Reise, 1994). The common periwinkle is native to European shores (Vermeij, 1982) but has shown to be present in a large variety of habitats and climates, ranging from Canada to Spain (Yamada, 1987). *L. littorea* has been used as a food source for centuries and still shows to have a large market for human consumption, particularly in France (Cummins et al., 2002). As in some cases, demand exceeds the local harvest, the need has been expressed to develop strategies to cultivate *L. littorea* (Castelo Branco et al., 2014). As cultivation of larval stages can be very labor intensive and require careful conditions (Castelo Branco et al., 2014), additional research efforts into the feasibility of on-growing techniques could already have a significant impact on the market and industry (Cummins et al., 2002). On-growing techniques could realize rapid growth rates, so the organisms can be cultivated in a profitable time period (Cummins et al., 2002). Cashmore & Burton (1998) show that growth rates of *L. littorea* in aquaculture facilities were significantly higher than growth rates of natural populations. In addition, they found that the highest growth rates occurred when the snails fed on *Ulva sp.* biomass. Therefore, exploring the co-culture of *Ulva sp.* and *L. littorea* may offer interesting opportunities.

1.6 Aim of Study

The goal of this study was to examine the potential of *Ulva sp*. cultivated in RAS wastewater, characterized by the high NO_3^- levels, and how the growth, chemical composition and uptake performance were affected by this medium. To research the prospective of *Ulva sp.* in this high NO_3^- rich medium, a cultivation system was established where *Ulva sp.* was continuously supplied with RAS water that had received biological and mechanical filter treatments. This system was used over a period of 27 days to find the effects on the seaweed biomass. To test the strength of the effect by the RAS wastewater, different RAS concentrations between 100 and 25% were used. It was hypothesized that the increased and concentrated nutrient availability of this waste stream positively alters the growth and the chemical composition of *Ulva sp*.. It was further hypothesized that different concentrations of RAS influence this effect, increasing in strength in higher RAS concentrations. In addition, this experiment examined the uptake response of *Ulva sp.* to the nutrient availability of the RAS waste stream with no water exchange. As was mentioned, the high NO_3^- levels in RAS wastewater may negatively alter the uptake and performance of the seaweed due to their preference for NH_4^+. Here, attention was paid specifically to the NO_3^- uptake in comparison with the NH_4^+. In addition, it was tested if

increasing the biomass density influenced the uptake behavior and removal efficiency. It was hypothesized that *Ulva sp.* successfully takes up the nutrients from RAS waste stream. Moreover, it was assumed that the *Ulva sp.* prefers NH_4^+ over NO_3^- , which should be visible by a higher specific uptake rate and a higher removal efficiency. In this experiment, it was also tested how the biomass density affects the uptake and removal efficiency. It was hypothesized that the uptake rate decreased with increasing seaweed biomass and the removal efficiency increased with increasing biomass.

This altered chemical composition and growth rate increase the suitability of *Ulva sp.* to be used as macro-algivore feed. To translate this potential, a co-cultivation system of *L. littorea* and *Ulva sp*. in an existing RAS system was simulated. To develop the co-culture of the *Ulva sp.* and *L. littorea* biomass, data on parameters for on growing of *L. littorea* were gathered by SINTEF Ocean. A trial was executed in which the effects of temperature and size were evaluated in the grazing and growth rate of the *L. littorea*. Here, it was hypothesized that increasing temperature has a positive effect on growth and grazing due to their poikilothermic status (Dehnel, 1995; Frick et al., 2018). The effect of size was hypothesized to negatively affect growth, but positively affect grazing. The data of the snail and seaweed experiments were combined to research the perspective of a co-culture of the two species in a RAS waste stream. The explorative model translated the cultivation potential of *Ulva sp.* in RAS waste streams into utilization of the additional biomass as macroalgivore feed. Additionally, the explorative model provided insights into what the desired biomass amounts should be for both species to exist in a balanced state of harvest, grazing and growth when cultivated in RAS wastestream.

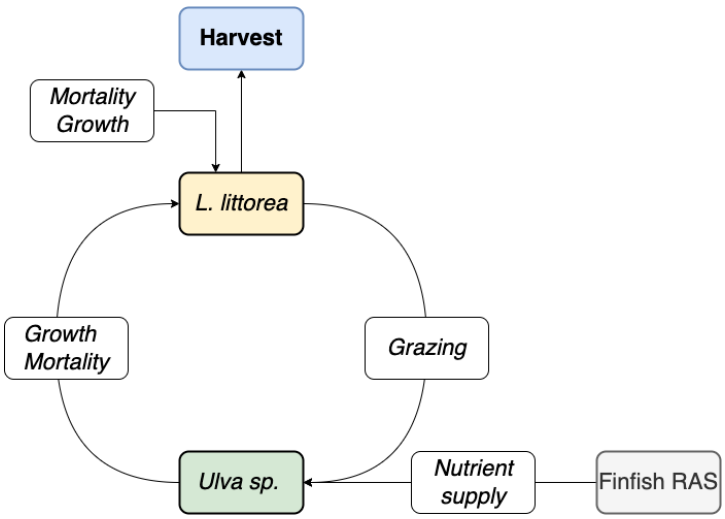

Figure 1. Simplified version of co-coculture of the *Littorina littorea* and *Ulva sp.* biomass in Recirculating Aquaculture System (RAS) wastewater.

2. Methodology and Materials

2.1 Cultivation of seaweed

Collection of Materials

For this experiment, the biomass of *Ulva sp.* was used. As it was not certain what the exact species was, the species was denoted as *Ulva sp.* throughout the whole study. The collection of *Ulva sp.* biomass was executed by a third party in the area of Valsholam, Trøndelag, Norway. The *Ulva sp.* biomass was manually wild harvested at low tide.

Set-up of Studies

The study was conducted in the laboratories of NTNU Sealab & SINTEF Ocean, Trondheim. The RAS water was received from Lerøy Midt, Hellandsjøen in Norway. This RAS facility produces salmon smolt (*Salmo salar*) and uses fresh water. The received RAS water was therefore increased in salinity to 15ppt using evaporated red sea salt from Red Sea Fish Pharm Ltd (Red Sea, n.d.). As this salt is evaporated seawater, the exact composition may vary per product and was before adding unknown.

A climate-controlled room was used, where the temperature was set to 13 °C. The bottles were aerated with a mix of air and added CO_2. To ensure that the air was optimally distributed through the bottle, one end of bottle was put slightly at an incline by placing a plastic test vile with a radium of approximately 1 cm under the bottle. By doing this, it was ensured that the biomass was kept in suspension. The addition of CO_2 was dependent on the pH in the bottles and so varied throughout the experiment. The aim was to keep the pH stable between 7.0 and 8.0. The pH was measured using a WTW pH 3210 measurement tool at an interval of approximately 3 days. Depending on the average pH changes in the bottles, the amount of added CO_2 was altered. The CO_2 was measured using an Extech instruments CO_2 meter (model CO250) giving values in parts per million (ppm). The average ppm during the experiment was approximately 690 ppm, with a range from 400 to 840 ppm. The biomass was illuminated from behind using white TL light. In the current experiment, light was measured using a WA12 ULM 500 universal light meter, measuring on the left outside of the bottles in the middle of the length and depth of the water. The appropriate light intensity was determined to be between 50-80 $\mu mol\ m^{-2}\ s^{-1}$ based on a pilot study conducted earlier in the year (May 2021). The pilot study was described in Appendix A.

The seaweed experiment was split up into two separate experiments, with different treatments and management but the above-described parameters were kept similar for both experiments. Seaweed Experiment I was focused on measuring the effects of the RAS water on growth and chemical composition. Seaweed Experiment II was focused on measuring the long-term uptake of *Ulva sp.* in RAS medium. In total, 32 bottles (VWR tissue culture flasks, 300 cm^2) were deployed to test six different treatments and two controls (Figure 2). Thus, every treatment and control had four replicates. For both Seaweed Experiment I and II, the controls contained artificial seawater made with distilled water and Red Sea salt from Red Sea Fish Pharm Ltd.. It was chosen to use this salt, as it contains levels of foundation elements similar to natural seawater (Red Sea, n.d.) and so mimics natural conditions for the *Ulva sp.* in the control. In both Seaweed Experiments, the controls contained 0.125 g L^{-1} *Ulva sp.*.
The cultivation medium and biomass density in the remaining bottles were determined by the treatments in the Seaweed Experiments and were described below.

Seaweed Experiment I was used to test to if the growth and chemical composition was altered when the biomass was cultivated in varying concentrations of RAS medium. To test the strength of the effect by the RAS wastewater, three different treatments and one control treatment were applied with different ratios between RAS water and artificial seawater (AS). The treatments contained 100%, 50 % and 25% RAS water and the control contained 100% AS. To mimic a RAS system, the cultivation media was renewed every three days.
The biomass in every bottle was similar and kept at the stable level 0.125 g L^{-1} throughout the whole experiment. To ensure that biomass was kept at this level, the growth of the biomass above this threshold would be removed when biomass registrations took place. The parameters measured in the first experiment can be found in Table 1.

The second experiment was used to research the long-term uptake of nutrients by *Ulva sp.* in RAS medium. As treatments, different densities of *Ulva sp.* were applied and uptake of NH_4^+, NO_2^-, NO_3^- and PO_4^{3-} was measured over an increasing time interval. The different biomass densities that were applied included 100% biomass (0.125 g FW L^{-1}), 200% biomass (0.250 g FW L^{-1}) and 300% biomass (0.375 g FW L^{-1}). As for experiment II there was no water renewal, more water evaporated during the experiment. To keep the water level and pH stable in this experiment, every three days the distilled water was added to the cultivation up to a mark that indicated 250 ml. As this measure included manual measurements, precision was expected to

be lower for the exact water level. The set-up and parameters measured in the second experiment can be found in Table 2.

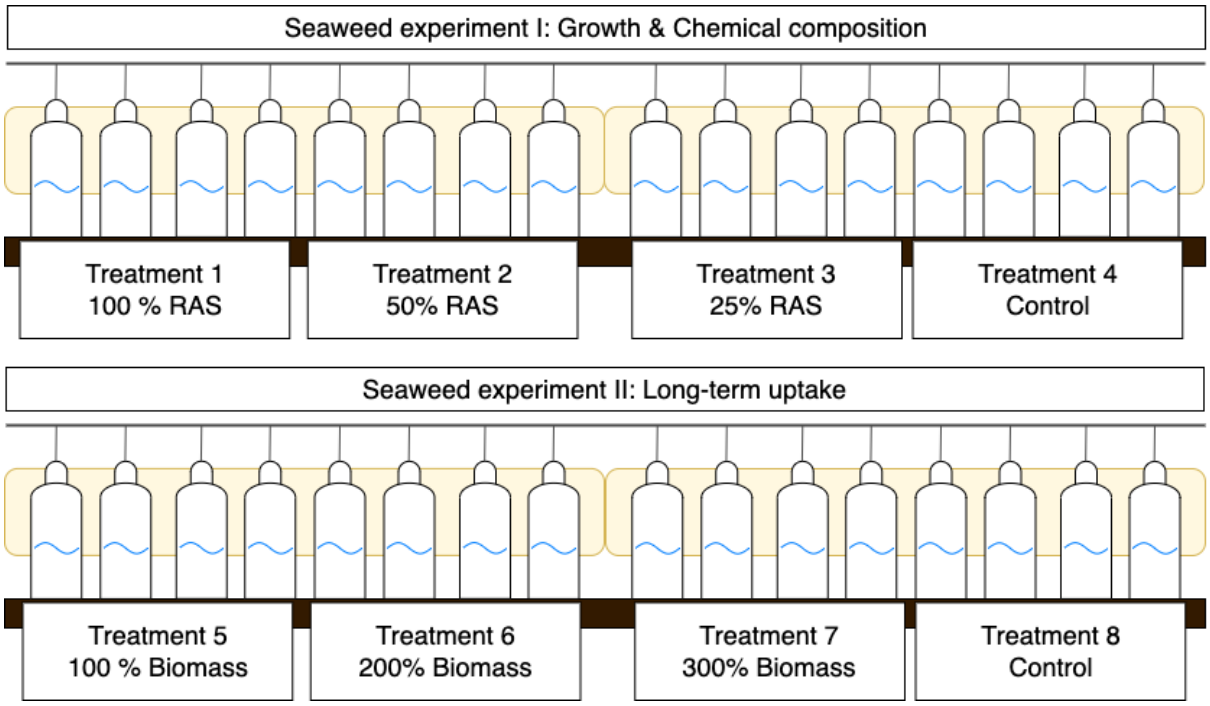

Figure 2: Set up of *Ulva sp.* experiments: Seaweed Experiment I and Seaweed Experiment II. The bottles containing the seaweed are illustrated by the white bottle shaped rectangles. The yellow rectangles behind the bottles indicate the light source.

Table 1. Overview of treatments employed in the Seaweed Experiment

	EXPERIMENT I	**EXPERIMENT II**
	GENERAL	
TEMPERATURE	13 °C	13 °C
VOLUME	250 ml	250 ml
PH	7.0-8.0	7.0-8.0
LIGHT	50-80 µmol m^{-2} s^{-1}	50-80 µmol m^{-2} s^{-1}
SALINITY	15 ppt	15 ppt
	MANAGEMENT	
TREATMENTS	100% RAS 50% RAS + 50 % AS 25% RAS + 75 % AS 100 % AS	5. 100% Biomass + RAS water 6. 200% Biomass + RAS water 7. 300% Biomass + RAS water 8. 100% Biomass + AS water
BIOMASS	0.125 g FW L^{-1}	See above
WATER MEDIUM	See above	RAS & AS (treatment 8)
WATER RENEWAL	33.33 % day^{-1}	No water renewal
DURATION	27 days	7 days
SAMPLE TAKING	Approx. every 6 days	0, 24, 72 and 168 h
SAMPLES TAKEN	Biomass registrations, Tissue	Water samples (4 ml)

Biomass Samples & Specific Growth Rate

For Experiment I, biomass registrations were taken approximately every 6 days to calculate the specific growth rate of the seaweed. When the biomass was over the desired level of 0.125 g L^{-1}, additional biomass was removed. The moment of registrations overlapped with the water renewal days, to ensure that the disturbance of the biomass was kept to a minimum. When the medium was renewed, the seaweed was easily taken out of the bottle, blotted dry with clean tissue and weighted on Mettler Toledo (g) scale. During biomass registrations, tissue samples would only be taken when the thalli had grown plenty to sample from. It would be deemed sufficient to sample from when the thallus had grown more than 10 g. When this was not possible, the seaweed would only be weighed. Sampled biomass was directly frozen (-19 °C) afterwards. In total, sampling occurred on day 0, 6, 9, 15, 21 and 27.

In Experiment II, seaweed was weighed at the beginning and end of the experiment. Measuring growth rate was not a goal of this experiment and was therefore only used to adjust the specific uptake rate so that it included the growth of the biomass. Therefore, the biomass registration took place on day 0 and day 27.

Using Eq. 1, the specific growth rate (SGR) in experiment was calculated as percental increase in weight (Ale et al., 2011; Ben Ari et al., 2014). Here, W_{t-1} was the wet weight of the specimen in grams at the previous sampling moment and W_t was the wet weight of the specimen in grams at the given sampling moment. It was chosen here to use W_{t-1} rather than W_0, as biomass was removed during every sampling moment. This removed biomass that could have contributed to the growth during the other sampling moments.

$$Eq.1 \qquad SGR\ (\%\ \ day^{-1}) = 100 * \ln \frac{\left(\frac{W_t\ (g)}{W_{t-1}(g)}\right)}{t_t(d) - t_{t-1}\ (d)}$$

Water Analyses & Uptake rate

For Experiment I, water samples were taken to test if the RAS treatments did not show any changes over time. The two time points, being 10/10/21 and 14/10/21, were randomly selected from the collection of water samples. The sample was filtered using a syringe and syringe filter (25 mm syringe filter with 0.45 µm CAM, VWR international, USA) to ensure no biomass was left in the water sample. The sample was stored in 15 ml sample tubes and directly frozen (-19°C) afterwards. For Experiment II, water samples were taken during the experiment with an increasing time interval. This included the null sample, 24 h, 72 h and 168 h. These time points

are hereafter referred to day 1, day 3 and day 7. When sampling, 4 ml of water was taken out the bottles during every sample moment. The sample was treated identically to water samples taken in Experiment I.

The water samples taken in both Experiment I and Experiment II were thawed and diluted according to the nutrient detection limits of photometric autoanalyzer (Flow solution IV System, O.I Analytical). The limits are 0.02-40 mol L^{-1}, 0.009 umol L^{-1} and 0.002 mg L^{-1} for NO_3-N, PO_4-P and NH_4^+N, respectively. Dilutions are summarized in Table 2. The results of the autoanalyzer were given in µg L^{-1} using a standard curve for the estimation. The margin of error here is ± 2 µg L^{-1}. The Norwegian Standards of NS4745 and NS-EN-ISO6878 were used for determination of NO_3-N and PO_4-P, respectively. Ammonium content was determined using the method described by Kéroul & Amoniot (1997).

Table 2. Dilutions for the nutrient analyzed in Seaweed Experiment I & II

NUTRIENT	NUTRIENT VALUE FROM LERØY SMOLT	NUTRIENT LIMIT FOR AUTOANALYZER	DILUTION RATIO (SAMPLE:DESTILLED WATER)
NH_4^+	460 µg L^{-1}	80 µg L^{-1}	1:5
NO_2^-	n.a.	350 µg L^{-1}	1:2
NO_3^-	178800 µg L^{-1}	350 µg L^{-1}	1:599
PO_4^{3-}	2000 µg L^{-1}	250 µg L^{-1}	1:7

For Seaweed Experiment II, the specific uptake rate (V) was calculated using Eq. 3, a formula adapted from Forbord et al. (2021). To ensure that the growth of the seaweed was incorporated, this was included in the calculation. Using Eq. 2, the growth rate per treatment was calculated and applied in Eq. 3. The specific uptake rate (*V*) was calculated for day 7. Lastly, using Eq. 4, the removal efficiency was calculated.

$$Eq.2 \qquad Growth\ rate\ = \left(\frac{Sample\ weight_t}{Sample\ weight_0}\right)^{1/t}$$

$$Eq.3 \qquad V\ (\mu g\ \ g\ DW^{-1}\ d^{-1}) = \frac{(Concenctration_0\ (\mu g\ l^{-1}) - Concentration_t\ (\mu g\ l^{-1})) * Bottle\ volume\ (L)}{Sample\ weight\ (\ g\ DW) * growth\ rate\ exp.II^{t(d)}}$$

$$Eq.4 \qquad Removal\ efficiency\ (\%) =$$

$$\frac{(Concenctration_0\ (\mu g\ l^{-1}) - Concentration_t\ (\mu g\ l^{-1}))}{(Concenctration_0\ (\mu g\ l^{-1}))} * 100\ \%$$

Experiment I: Tissue Analyses

Fresh Weight to Dry Weight ratio

To determine the ratio between fresh weight and dry weight for the *Ulva sp.* biomass, 20 samples of *Ulva sp.* biomass of varying weight were taken. The range was between 0.2 g and 3.79 g FW, with an average of 1.05 g. The samples were weighted, placed on an aluminum surface and dried from 24 hours at 60 °C using a Termaks KB 4000 oven. Afterwards, the samples were weighted again and the ratio DW:FW was determined using the following formula (Eq. 5):

$$Eq.5 \qquad DW:FW = \frac{1}{n} * \sum \frac{Weight\ seaweed\ before\ drying\ (g)}{Weight\ seaweed\ after\ drying\ (g)}$$

For the tissue analyses, all the samples were first freeze dried using Labronco Freezone (8L) for a period of 24 hours. The period of 24 hours was determined by a test conducted before to see what the appropriate drying time was. Afterwards, the freeze-dried sample of *Ulva sp.* was blended into little shards.

Carbon Tissue Content, Nitrogen Tissue Content and C:N Ratio

Samples of on average 2.3 mg freeze dried *Ulva sp.* were weighed (Mettler Toledo UMT2), packed into capsules (5x9 mm) and wrapped into small cubes on a carbon free metallic plate. Prior to the C:N analyses, the small cubes were dried at 60 °C. Acetanilide was used as standard on an elemental analyzer to determine the carbon and nitrogen content of the sample (Elementar vario EL cube, Elementar Americas Inc., New York). Using Eq. 6, the tissue content was calculated. Following this, the percentage of nutrient content in DW seaweed was calculated using Eq. 7. These nutrient contents were used to calculate the C:N ratio, which was the main aim of this analyses. Tissue ratios of C:N were established by using Eq. 8.

$$Eq.6 \qquad Tissue\ content\ (\mu g\ mg\ DW^{-1}) = \frac{Weight\ of\ nutrient\ (\mu g)}{Weight\ of\ capsule\ (mg\ DW)}$$

$$Eq.7 \qquad \%\ of\ DW = \frac{Tissue\ content\ (\mu g\ \ mg\ DW^{-1})}{Weight\ of\ sample\ (\ mg\ DW^{-1}) * 1000\ \mu g}$$

$$Eq.8 \qquad C:N = \frac{Weight\ of\ carbon\ (\mu g\ C\ mg\ DW^{-1})}{Weight\ of\ nitrogen\ (\mu g\ N\ mg\ DW^{-1})}$$

Protein Content

Then, the nitrogen content was used to calculate the protein content. Using the total nitrogen content (TN) and a protein conversion rate specifically for green algae, the protein content was analyzed in the tissue samples. As plants and algae contain non-protein nitrogenous material, the common conversion factor of 6.25 often leads to an overestimation of protein value (Angell et al., 2016). Based on the publications of Angell et el. (2016) and Biancarosa et al. (2017) that found a green algae specific conversion factor of 4.24 and 4.49 respectively, the average of those two was taken. The protein content was calculated using Eq. 9.

$$Eq.9 \qquad Protein\ content\ (\%) = 4.36 * tissue\ content\ N\ (mg\ 100\ mg\ DW^{-1}) * 100\ \%$$

Organic Phosphate

The organic phosphate content was determined using the method described by Koroleff (1976). Samples of the freeze-dried *Ulva sp.* were weighed (Mettler Toledo UMT2) and put into marked plastic bottles. To ensure that the organic phosphorous was detected, the particulate organic phosphorous samples were converted to dissolved inorganic phosphorous. This was done by adding 10 ml of distilled H_20, 2ml of oxidizing reagent (50g $K_2S_2O_8$ L^{-1} dH_2O) and 0.1 ml of acid (4M H_2SO_4). The bottles were then autoclaved for 30 minutes at 120 °C. Detection of nutrient was done following the NS-EN-ISO6878 method on an autoanalyzer (Flow Solution IV System, O.I. Analytical). The phosphorous tissue content was calculated using Eq. 10. In addition, the N:P ratio was calculated using Eq. 11.

$$Eq.10 \qquad Tissue\ content\ P\ (\mu g\ P\ mg\ DW^{-1}) = \frac{Measured\ content\ (\mu g\ P\ L^{-1}) * Sample\ solution\ volume\ (L)}{Sample\ weight\ (\ mg\ DW)}$$

$$Eq.11 \qquad N{:}P = \frac{Weight\ of\ nitrogen\ (\mu g\ N\ mg\ DW^{-1})}{Weight\ of\ phosphorous\ (\mu g\ \ P\ mg\ DW^{-1})}$$

Statistical Analyses

Data was analyzed with R Studio (version 4.0.2 (2020-06-22) -- "Taking Off Again"). Aside from the standard packages that are included when downloading R, additional packages that were used in the analyses and plotting of data included 'ggplot2', 'ggpubr', 'gridExtra', 'multcompView', 'Rmisc' and 'sciplot'. Data from Seaweed Experiment I and II were fitted in a linear model and an analysis of variance (ANOVA). Where possible, also a post hoc Tukey test (Tukey) was performed.

To test the fit of the model, it was plotted using the function plot('model') to see if the model meets the requirements for a linear model. The analytical plots that included are (1) Residuals vs fitted, (2) Normal QQ, (3) Scale-Location and (4) Residuals vs leverage. The requirements that needed to be met with these plots include (1) no heteroscedasticity or patterns, (2) approximately a line of 45 degrees, (3) an approximate equal spread of residuals along range of predictors and a horizontal red line and (4) no data points outside of the red dotted lines. In addition, a histogram of the residuals was plotted to see if the data is normally distributed.

The response and predictor variables that are included in these models are in Table 3. In Experiment I and II, the variable of treatment was factorized and resulting in twice a factor with four levels. For Experiment I, this was level 1-4; for experiment II level 5-8 were used to indicate the different RAS and density treatments (see Table 1). In experiment I, for the SGR model, the variable of days was treated as a continuous variable. However, for the tissue analysis of experiment I, the variable was treated as factorized variable with three. This change in characterization of the variable was caused by the difference in number of samples that were taken. If the variable of days contained more than 3 measurements, than it was considered as continuous. In models where possible, an interaction effect was included between treatment and days, as it was expected that the effect of the treatments was strengthened over time. For all models developed, the significance level was 0.05.

Table 3. Response and predictor variables for Seaweed Experiment s

EXPERIMENT	RESPONSE VARIABLE	PREDICTOR VARIABLES
EXP. I	SGR C:N & N:P Protein Tissue content (C, N, P) % C, N, P of tissue weight	Light, pH, Days, Treatment
EXP. II	Specific uptake rate Removal efficiency Nutrients left in beaker	

First, all parameters were included in the model (see Table 3). The selection of which model to use, i.e., which parameters were included, was based on the AIC score of the model. The model with the lowest score was selected.

For some measurements moments, a proxy of pH and light had to when data on the sampling day were missing (Table 4). In addition, for some of the null samples with the control water, a proxy of previous measurements had to be taken as the null sample of the artificial seawater was missing.

Table 4. Overview of proxies used in Seaweed Experiment I and II

MODEL	VARIABLE	MISSING MEASUREMENT	PROXY USED
EXP I: SGR & TISSUE	pH	Day 6	An average of day 5 and day 7
EXP I: SGR & TISSUE	pH	Day 15	An average of day 14 and day 18
EXP II: UPTAKE	pH	Day 1	Value used of day 0
EXP II: UPTAKE	pH	Day 3	An average of day 2 and day 3
EXP II: UPTAKE	NH_4^+ NO_2^- NO_3^- PO_4^{3-}	Null sample	Average of samples day 3 and 7

2.2 Snail Experiments and calculations

Collection of Materials

The collection of *Littorina littorea* was executed by a third party, in the area of Ørland kommune, Trøndelag, Norway. The *L. littorea* biomass was manually wild harvested without the use of scuba gear.

Table 5. Treatments applied in the Snail Experiment.

TEMP (°C)	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**
	8.6	9.5	10.8	11.8	13.1	14.3	15.2	16.2	17.4	18.4
					Size group					
1 (6 mm)	T1-1	T2-1	T3-1	T4-1	T5-1	T6-1	T7-1	T8-1	T9-1	T10-1
2 (8 mm)	T1-2	T2-2	T3-2	T4-2	T5-2	T6-2	T7-2	T8-2	T9-2	T10-2
3 (10 mm)	T1-3	T2-3	T3-3	T4-3	T5-3	T6-3	T7-3	T8-3	T9-3	T10-3
4 (12 mm)	T1-4	T2-4	T3-4	T4-4	T5-4	T6-4	T7-4	T8-4	T9-4	T10-4
5 (14 mm)	T1-5	T2-5	T3-5	T4-5	T5-5	T6-5	T7-5	T8-5	T9-5	T10-5

Set-up of Study

The set up and execution of the study was done by SINTEF Ocean in the period of April to June 2021. The data were received after the completion of the experiment. SINTEF Ocean conducted a snail growth and a snail grazing experiment, both of varying durations. The snail growth experiment lasted for 63 days with measurements taken on day 0, 31 and 63. The grazing experiment was executed twice; once on day 1-4 and once on day 57-60. In total, ten temperature treatments between 8.6 and 18.4 °C were deployed to test the effect of temperature on growth and grazing exhibited by the snails (Table 5). Every treatment contained five snails of different sizes. The snails were sorted according to size and the sizes that were included in the experiment were 6, 8, 10 ,12 and 14 mm. Therefore, every treatment contained snails of the five size categories. During the study, pH, salinity and dissolved oxygen were measured and included in the analyses.

Collection of Samples & Formulas

To calculate the SGR as percental increase snail's growth, the data for the shell height was used, as this is the most common way to measure growth (Cummins et al., 2002). Shell height was calculated by measuring the distance between the protoconch to aperture. The percental increase in height as SGR is shown in Eq. 12.

$$Eq.12 \qquad SGR\ (\%\ day^{-1}) = 100 * \ln\frac{\left(\frac{H_t\ (mm)}{H_0(mm)}\right)}{t}$$

To calculate the consumption of *Ulva sp.* biomass by *L. littorea* in a cultivation system, grazing rate was calculated by the amount (g) of *Ulva sp.* that was eaten in a day (Eq. 13). Standardized disc (58.5 mm diameter) of *Ulva sp.* were used and weighed every day to measure grazing. To incorporate the growth of the seaweed that may occur in these days, growth rate data from the Experiment I was used. Eq. 9 was used to calculate these growth rates for Experiment I.

$$Eq.13 \qquad Grazing\ rate = (g\ \ day^{-1})$$

$$\frac{weight\ Ulva_t\ (g) - (weight\ Ulva\ _{t-1}(g)\ *\ growth\ rate\ )}{t\ (d)}$$

Statistical Analyses

By using the different sizes of snails as replicates per treatment by SINTEF Ocean, there are no true replicates present in the study. As it was expected that the different sizes of the snails are differently affected by the temperature ranges, no statistical tests can be executed. This is because the chosen statistical method of ANOVA and linear regression assume an equal probability per replicate to be affected by the treatment; this was not the case in this experiment. To still give an insight into the behavior of *L. littorea* in cultivation systems, mean values per treatment and per size class were calculated.

2.3 Explorative Model Development

System Boundaries

The data of the snail and seaweed experiments were combined for the development of the explorative model. The goal, as was defined in the introduction, was to gain insights into what the desired biomass amounts should be for both species to exist in a balances state of harvest, grazing and growth when cultivated in a RAS waste stream.

The system boundaries for the simulated co-culture have been shown in Figure 3. Below, these boundaries are described in more detail.

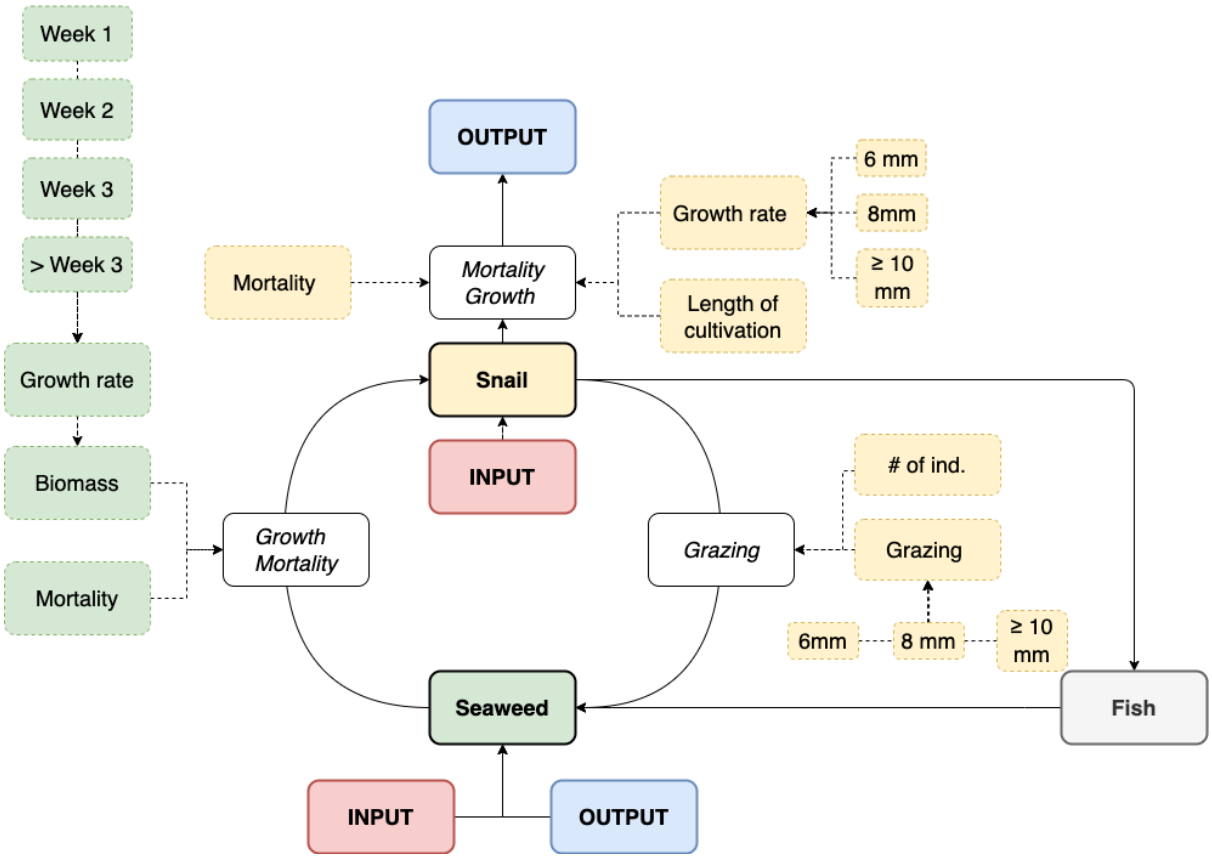

Figure 3: Overview of the explorative model

Most of the values that served as input into the explorative model were based on the seaweed and Snail Experiments. Some of the values needed to complete the explorative model were missing. These values were substituted with values that were found using a literature search with the search engine 'Google Scholar'.

The farm that this explorative model was based on, does not exist. Therefore, the following information should be considered as hypothetical. Based on information provided by the third

party, a hypothetical farm was the size of 2240 m^2 and the desired density of the snails was approximately 150 individuals per m^2. This was calculated on the basis that there was a 4 cm radius of space between each snail. The ideal size allocation between the snails and the seaweed was found by running different scenarios with different allocations of the total farm size available. The ideal size allocation was defined by the following parameters: (a) the snails can be harvested at a steady rate, (b) the snails can continuously graze on *Ulva sp.* biomass and (c) over a period of five years, the seaweed biomass does not need to be restocked. The focus of the explorative model was year based, meaning that every value, e.g., snails being harvested, or *Ulva sp.* grazed, was calculated on an annual basis. In total, the explorative model was run for 5 years.

Total Snail in- and output

First, based on Eq. 14, the total cultivation cycle, expressed in days, was found based on the size specific growth rates. This included size 6 to 12, as it was assumed that the snails entered the on-growing system at 6 mm and harvest occurred at 14 mm. The total cultivation cycle was calculated by summing the grow-out period, in days, that the snails of a certain size needed to reach to next snail size. Growth rates and grazing found during the experiments in treatment 5 are used, as this temperature was close to the temperature in which the *Ulva sp.* was cultivated. To find the total number of snails present in the system, the density and the size allocated in the farm area for the snails was used (Eq. 15). To consider the effect of mortality, this number was later multiplied with the survival rate of the snails.

$$Eq.14\ Total\ grow-out\ period(d) =$$

$$\sum \log_{growth\ rate\ size\ specific} \left(\frac{Size\ aim\ (mm)}{Current\ size\ (mm)}\right)$$

$$Eq.15\ Number\ of\ snails\ (ind) =$$

$$size\ allocation\ (m2) * density\ (ind\ m^{-2}) * (1 - mortality\ rate)$$

Total Seaweed in- and output

To estimate the seaweed growth and biomass in the system, the growth rates from the Seaweed Experiment I were used. The growth rates that were used are based on the findings in the Seaweed Experiment I and were applied to ensure that the growth in the system mimics the growth the seaweed exhibited in the seaweed exhibited. In addition, the snail grazing was taken

into account when calculating the total seaweed in the system. To find the consumption of *Ulva sp.* by the snails, the grazing rate was multiplied by the number of individuals (Eq. 16). The total grazing was calculated daily to ensure that it fitted with growth pattern shown by the *Ulva sp.* in the RAS medium. The grazing rate was calculated per size category.

Eq. 17 show how the seaweed growth and consumption were incorporated into the explorative model and was used to calculate the total seaweed biomass per given time period. The formula uses the seaweed surplus from the year before to calculate the total seaweed output at the end of the given year. However, for the first year, the seaweed start stocking density was used as there was no surplus on day 0.

$$Eq.16 \qquad Total\ \ grazing\ \ (kg\ \ \ day^{-1}) =$$

$$grazing\ rate\ size\ specific\ (kg\ day^{-1})\ *\ number\ of\ individuals$$

$$Eq.17 \quad Seaweed\ biomass\ \ (kg\ FW\ \ t_{i+1}) =$$

$$\big(Seaweed\ surplus\ previous\ year\ \ (kg\ FW\ \ t_{i-1}) * growth\ rate^{t} * (1 - mortality\ rate)\big) - grazing\ (kg\ {t_i}^{-1})$$

Assumptions

There are several assumptions that were included in this explorative model. First, as this is a preliminary model working with limited data and time, only averages were used that were found in the experiments or in articles. This means that the variation in the data was not taken into account. Furthermore, it also assumed that the NO_3^- values are not above the safe limits for the cultivation of *L. littorea*. Lastly, it also assumed that the growth rates for the snails and seaweed were representative for organisms in the RAS wastewater over the five-year period.

Robust scenarios

As has been stated above, this explorative model only worked with the averages that were found in the Snail and Seaweed Experiments. To consider that there may be variation in these growth and grazing rates of the snails and the seaweed, several robustness scenarios were taken into account. The robustness of the scenarios that were used, influence the growth rate of the seaweed, grazing rate of the snails and the mortality of both the snails and the seaweed.

3. Results

The data included in the results existed of three separate inputs: data collection from the Seaweed Experiments, data collection by SINTEF from the Snail Experiment and literature data for the explorative model. Below, all data sets and results were described in segments of Seaweed Experiment I and II, Snail Experiments and Model development.

When statistical analyses were performed, the models that were shown all meet the requirements of ANOVA and linear regression, as specified in the methodology. The model outputs can be found in the appendices. If a data set had to be altered to fit the requirements of the model better, it was mentioned in the text of the respective appendix.

3.1 Seaweed Experiment I: Growth & Chemical Composition

Growth

The data set contained 80 samples (n=80) that measured the SGR as the percental increase in weight of the seaweed. Firstly, the results showed that there are no significant differences among the SGR of the RAS treatments, however, all the RAS treatments did significantly differ from the control. The trend between the treatments and control is that the average SGR of the seaweed decreased with the decreasing concentration of RAS water, except for 25% RAS. This treatment had the highest SGR on average of 6.6 % compared to 6.1%, 5.5% and 1.8% $day^{-1}$for 100% RAS, 50% RAS and control, respectively. The SGR of the RAS treatments was 337% higher than the control.

Secondly, there was a significant effect of time (Days) on the SGR of the seaweed. This relationship between SGR and time was negative for all the RAS treatments, meaning that over time the SGR of the seaweed decreased (Figure 4). This negative relationship was that the largest in the highest RAS concentration, i.e., 100% RAS. This treatment showed the strongest decrease in SGR over time, as was shown by the largest negative slope (Table 6). Overall, the SGR of 25 % RAS treatment showed to be the most stable over time. This was illustrated by the smallest slope (Table 6). For all RAS treatments, the SGR of the seaweed declined over time and came close to the SGR shown by the seaweed in the control. The SGR values in the first week of the experiment averaged for all the RAS treatments was 8.4% and 2.3% day^{-1} for the control, showing to be 365% higher than the control. At the end of the experiment, the average SGR for the RAS treatments was 0.5% and for the control -0.6%. Then, the average SGR of the RAS treatments was only 83% higher. It should be noted that the 100% RAS, as

the only RAS treatment, did not display positive growth anymore but an SGR of -1.6% day^{-1}. At the end of the experiment, the SGR of the 25% RAS treatment was still the highest, with a value of 2.7 % day^{-1}. Overall, the model had an adjusted R^2 of 0.4 and a p value of 4.95e^{-07}.

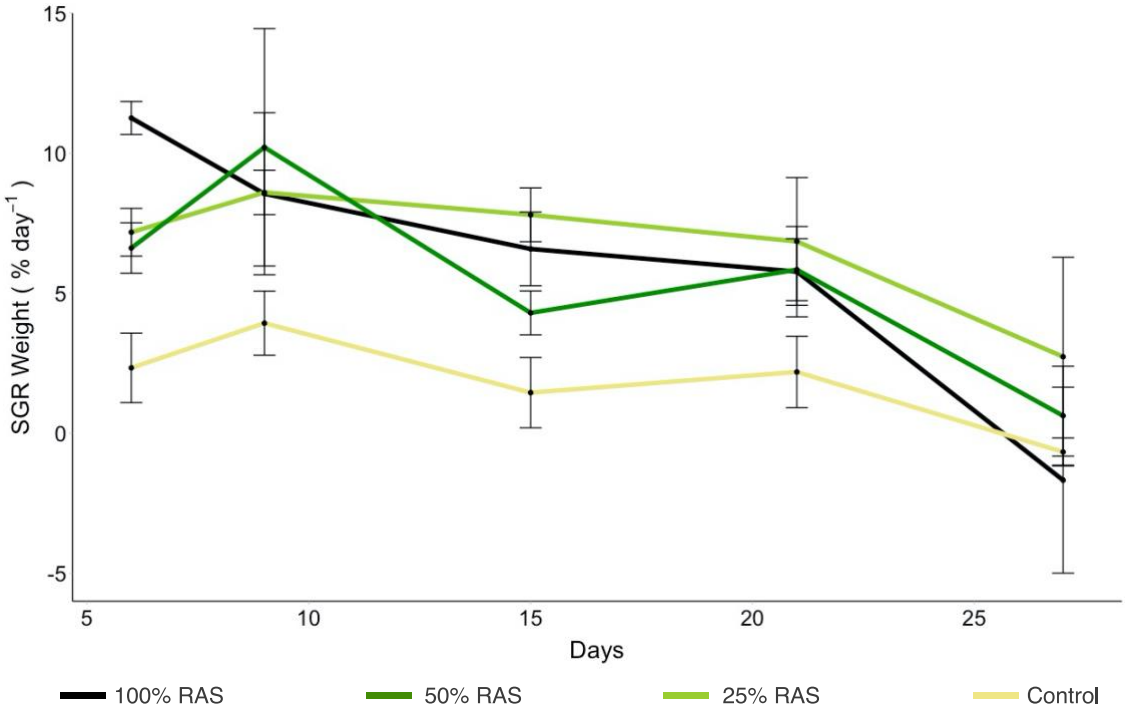

Figure 4. Specific growth rate (SGR) as percental increase in the weight of the *Ulva sp.* in Experiment I over the time period of 27 days. The data is grouped by Recycling Aquaculture System (RAS) Treatment, as displayed in the legend. Sampling took place on days 6, 9, 15, 21 and 27. The error bars in the graphs display the standard error with a significance level of 0.05.

Table 6: Slope, intercept, adjusted R^2 and p value for the SGR as percental increase in weight for the *Ulva sp.*. The data is grouped by Recycling Aquaculture System (RAS) Treatment or Artificial Seawater (AS) as the control. The stars in the right column denote the significance codes for the whole models as a function of the effect of time (days) on the SGR per specific treatment. The models are run with a significance level of 0.05. The stars denote the significance codes between the following values 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

	INTERCEPT	**SLOPE**	**ADJUSTED R^2**	**P VALUE**	**SIGNIFICANCE LEVEL**
TR. 100% RAS	14.36	-0.53	0.47	<0.001	***
TR. 50% RAS	10.52	-0.32	0.21	0.02	*
TR. 25% RAS	9.92	-0.21	0.11	0.08	
CONTROL: 100% AS	4.22	0.15	0.19	0.03	*

Tissue Analyses

The data set for the tissue analyses contained n=44 samples with samples from day 0, day 6 and day 27. This data was used for the analyses of the tissue content of carbon, nitrogen and organic phosphate. Additionally, the results for C:N and N:P were presented. It should be noted that on day 6 it was not possible to take samples from all the bottles, as some had not shown sufficient growth to remove biomass from. This applied to one bottle in treatment 25% RAS and two bottles in the control. Therefore, these treatments contained less replicates.

Carbon and Nitrogen content

The data for both the carbon and nitrogen content have been visualized in Figure 5 in µg mg DW^{-1}. Firstly, for the carbon content, there was a significant positive effect of the first week (day 6) compared to the null sample but not compared to the end of the experiment (day 27). Between day 6 and day 27, the values of carbon tissue content dropped, but were still slightly higher than the null sample. During the whole experiment, none of the RAS treatments and control significantly differed from each other, except for 50% RAS at the end of the experiment. At the end of the experiment, this treatment had a significantly higher carbon tissue content compared to the other treatments. Overall, the model had an adjusted R^2 of 0.58 and a p value of $1.504e^{-05}$.

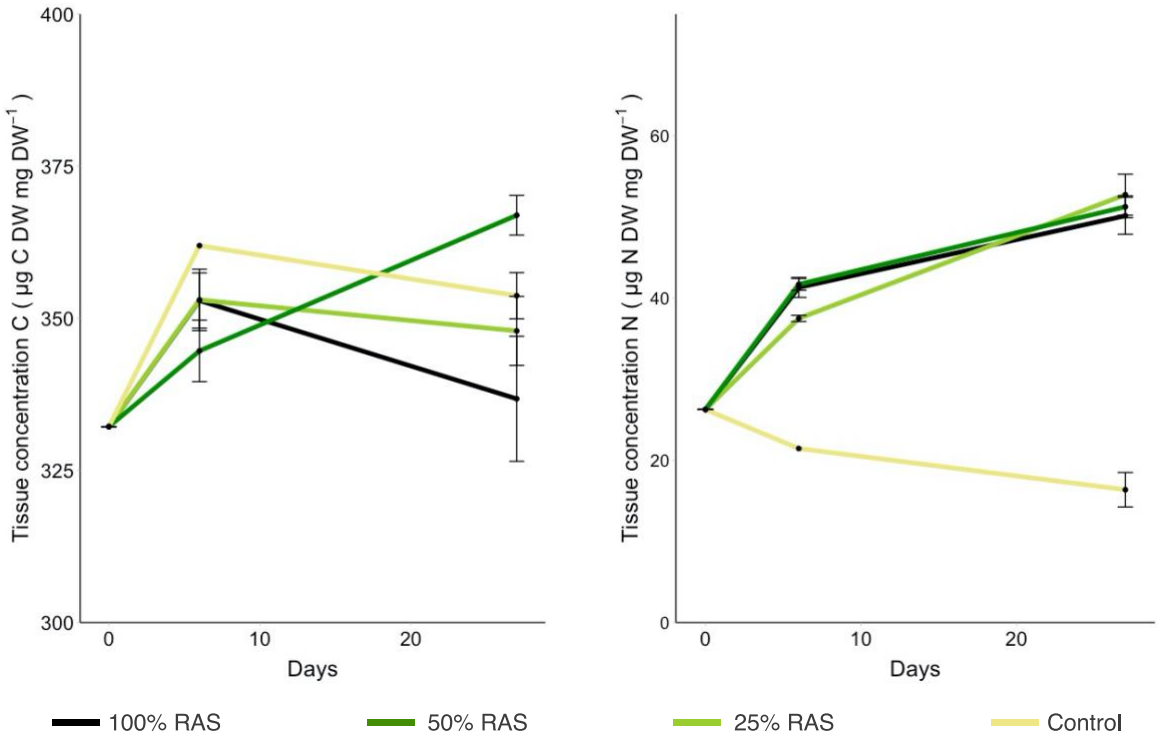

Figure 5. The tissue concentration (µg DW mg DW^{-1}) of carbon (C) and nitrogen (N) in the *Ulva sp.* biomass in Experiment I over the time period of 27 days. The data is grouped by Recycling Aquaculture System (RAS)

Treatment, as displayed in the legend. Sampling took place on days 6 and 27. The error bars in the graphs display the standard error with a significance level of 0.05.

Secondly, for the nitrogen content, there was a significant difference between the null sample and the RAS treatments in the first week (day 6) and the end of the experiment, showing an increase between every sampling period. The strongest increase in nitrogen tissue content occurred in the first week of the experiment compared to the end of the experiment for all the RAS treatments. In addition, none of the RAS treatments differed from each other over the whole testing period. All the RAS treatments showed a positive increase over the whole testing period, whereas the control showed a decrease over the whole testing period. Overall, the model had an adjusted R^2 of 0.95 and a p value of $<2.2e^{-16}$.

C:N

Based on the carbon and nitrogen content the C:N ratio was modelled and has been illustrated in Figure 9. Overall, the model had an adjusted R^2 of 0.86 and a p value of $9.17e^{-13}$. The model showed that both day 6 and day 27 had a significant effect on the C:N content of the seaweed, mirroring the trend compared of the nitrogen tissue content. Overall, the C:N ratio declined over time for all the RAS treatments. The strongest decline in C:N for the RAS treatments occurred in the first week of the experiment. During the experiment and at the end, the C:N ratio of the RAS treatments was significantly different from the C:N ratio of the control. In the end, the C:N ratio of the seaweed in RAS treatments was 8.6 and for the seaweed in the control, the C:N ratio was 22.5. The C:N ratio of the control was therefore 261% higher than the RAS treatments.

Protein content

Secondly, the protein content of the seaweed was analyzed and has been illustrated in Figure 6. The adjusted R^2 of this model was 0.93 and has a p value of less than $2.2e^{-16}$. The results of the protein content were similar to the results of the nitrogen tissue concentrations. As with the C:N ratio, day 6 and day 27 significantly affected the protein content of the seaweed. Overall, the highest protein content occurred on day 27. However, corresponding to the C:N results, the strongest increase occurred in the first week. Overall, the protein content was highest in the 25% RAS treatment at the end of the experiment. However, this difference was not significant. The only significant difference in this model was between the RAS treatments and the control on both day 6 and 27. In the end, the average protein value of the seaweed for the RAS

treatments and the control was 22.4 g 100 g DW^{-1} and 7.1 g 100 g DW^{-1}, respectively. The protein content of the RAS treatments was therefore 315% higher than the RAS treatments.

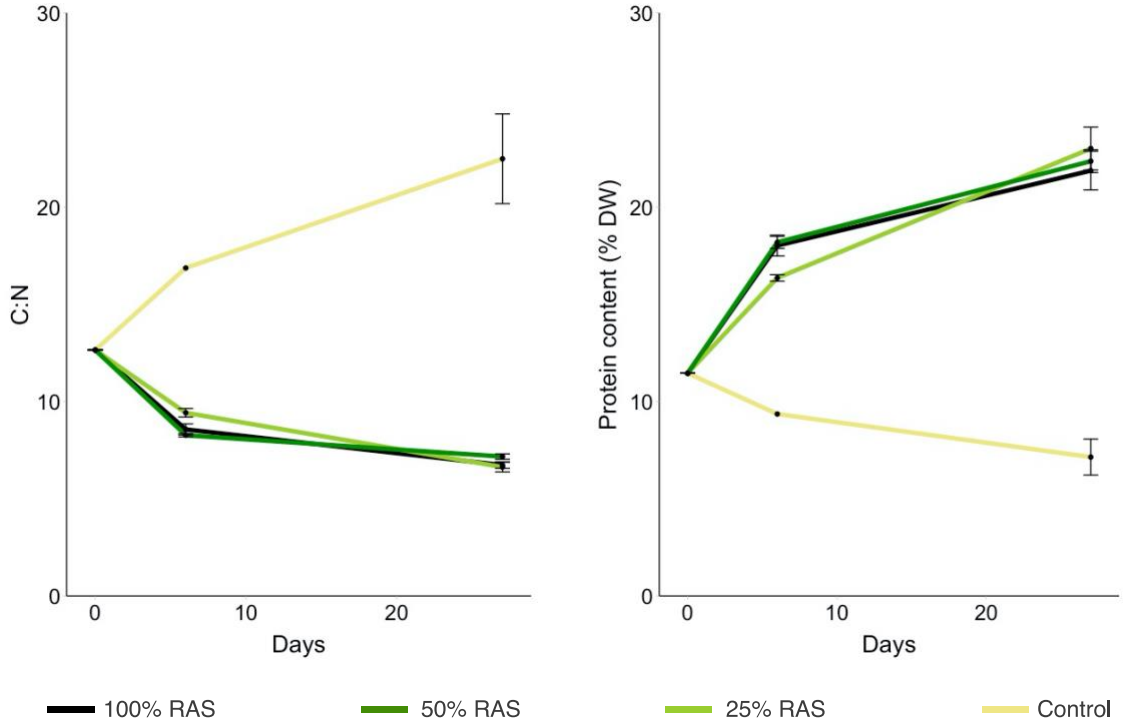

Figure 6. The C:N ratio and protein content (% of DW) in the *Ulva sp.* in Experiment I over the time period of 27 days. The data is grouped by Recycling Aquaculture System (RAS) Treatment, as displayed in the legend. Sampling took place on days 6 and 27. The error bars in the graphs display the standard error with a significance level of 0.05.

Organic Phosphate

Lastly, the organic phosphate content of the seaweed has been illustrated in Figure 7. The adjusted R^2 of this model was 0.73 with a p value of $1.71e^{-07}$. Like with the other tissue content analyses, there was a significant effect of time on both days and the strongest increase for the RAS treatments occurred in the first week. For the control, the strongest decrease occurred in this period as well. At the end of the experiment, the RAS treatments did not differ significantly from each other and all the RAS treatments showed an increased P tissue content compared to the beginning of the experiment. After one week of the experiment, the highest organic phosphate tissue content was highest in the 100% RAS treatment and lowest in 25% RAS treatment. However, at the end of the experiment the difference was shown to be non-significant. At the end of the experiment, the average organic phosphate tissue content for the RAS treatments and the control was 2 µg P mg DW^{-1} and 0.3 µg P mg DW^{-1}, respectively. The organic phosphate content was therefore 667% higher in the RAS treatments than in the control.

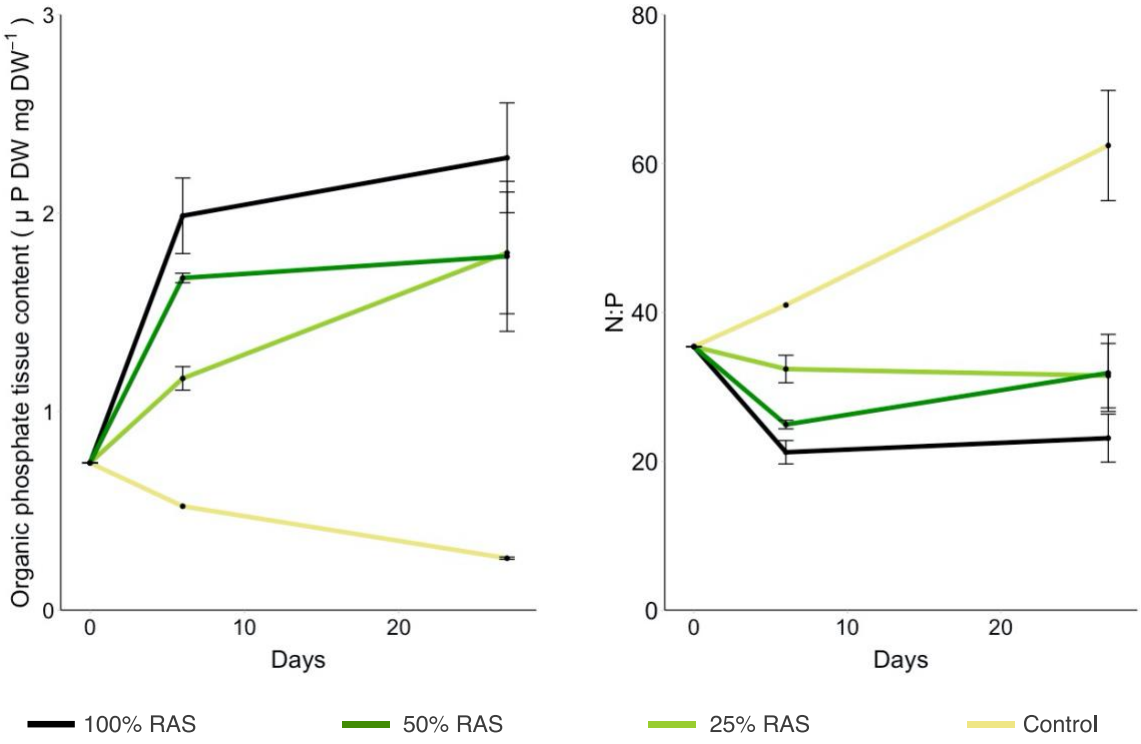

Figure 7. The organic phosphate content of the seaweed tissue (µg mg DW^{-1}) and the N:P in Experiment I over the time period of 27 days. The data is grouped by Recycling Aquaculture System (RAS) Treatment, as displayed in the legend. Sampling took place on days 6 and 27. The error bars in the graphs display the standard error with a significance level of 0.05.

N:P

For the N:P ratio, correspondingly to the P tissue content, there showed to be a significant effect of days for all treatments on the N:P ratio. The interaction between time and treatments was significantly different between RAS treatments and control and showed a strong increasing trend over time. At the end of the experiment, the RAS treatments were not significantly different from each other. Then, the average N:P ratio for the RAS treatments and control was 28.2 and 62.4, respectively. Therefore, the N:P ratio was 221 % higher in the RAS treatments than the control. Overall, the adjusted R^2 of the model was 0.70 and the p value was $1.87e^{-07}$. The results have been illustrated in Figure 7 above.

Water stability

In addition, the nutrient levels in the water were tested to see if these were similar during the experiment. The dates that were randomly selected occurred on a day before water renewal would take place. In contrast, the null sample was taken before the seaweed was put in. The

nutrient levels for the sampling moments have been summarized in Table 7. Overall, these results showed that there was a difference between the null sample and the two random sample moments during the experiment, showing the uptake of nutrients by the seaweed. As there was no significant difference between the sampling moments during the experiments, it can be expected that the RAS water was relatively stable.

For NH_4^+, there was no significance difference between the treatments. For NO_2^-, there was only no significant difference between treatment 100% RAS & treatment 50% RAS and treatment 25% RAS & control. For NO_3^-, there was no difference among the first three treatments, but they all significantly differ from control. For PO_4^{3-}, all the treatments significantly differed from each other, except treatment 50% control and 25%. All the results of the post hoc tests have been shown in Table 12.

Table 7. Significance levels of the post hoc Tukey test between Recycling Aquaculture Treatments (RAS) treatments and artificial seawater (AS) control of water samples from experiment I. The stars in the right column denote the significance codes between: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. The post-hoc Tukey test was run with the significance level of 0.05. Additionally, separate linear regression models were run with the separate nutrients to find the adjusted R^2 with the significance level of 0.05.

	NH_4^+	***NO_2^-***	***NO_3^-***	***PO_4^{3-}***
		DAYS		
NULL – 10/10	<0.001 (***)	0.0184	<0.001 (***)	<0.001 (***)
NULL – 14/10	<0.001 (***)	<0.001 (***)	<0.001 (***)	<0.001 (***)
14/10 – 10/10	0.800	0.254	0.915	0.701
		TREATMENTS		
50% RAS-100% RAS	0.991	0.350	0.975	0.005 (*)
25% RAS-100% RAS	0.823	0.018 (*)	0.868	0.001(**)
CONTROL-100% RAS	0.600	<0.001 (***)	0.000 (***)	<0.001 (***)
50% RAS-25% RAS	0.941	0.504	0.985	0.917
CONTROL-50% RAS	0.770	0.002 (**)	0.000 (***)	<0.001 (***)
CONTROL-25% RAS	0.980	0.086	0..000 (***)	0.002 (**)
ADJUSTED R^2	0.47	0.75	0.51	0.76

3.2 Seaweed Experiment II

This data set was split up in four sub data sets, each of them containing one of the nutrients. All data sets contained n=48 samples. The response variable was the specific uptake rate (V) (µg g DW^{-1} day^{-1}), removal efficiency (%) and nutrient concentration (µg L^{-1}) in the water.

For the display of the data of the specific uptake rate and removal efficiency, no trend line was provided as the variable of days has been factorized. Thus, there was no continuous variable present.

NH_4^+

The results for specific uptake rate (V) of NH_4^+ have been plotted in Figure 8. All treatments, including the artificial seawater, showed a significant difference in V compared to treatment with 100% biomass. Between treatment 200% biomass, 300% biomass and control there were no significant differences. Compared to the treatment with 100% biomass, the other treatments showed a decline in V, with a slight trend visible of declining V with increasing biomass. This trend, however, was not significant. The lowest V was found in the control with artificial seawater. The overall adjusted R^2 of this model was 0.67 with a p value of <0.0001.

The removal efficiency has also been illustrated in Figure 8. As can be seen, the removal efficiency for all the RAS treatments was not significantly different from each other, only from the control. The highest removal efficiency was shown in treatment 7 with 300% biomass, showing a removal efficiency of approximately 36%. There did not seem to be a trend of visible in the data with increasing biomass density.

When looking at the nutrient concentration of the water in Figure 11, it showed that the nutrient concentration between the experiment days varied. From day 0 to day 1, there was a strong decline in the nutrient value. Between day 1 and day 3, this trend was continued, however showed to be less strong. After day 3, the NH_4^+ showed an increase. Additionally, it was visible that the nutrient concentrations of all treatments, including the control, were all very similar at the end of the experiment. This was confirmed by the statistical model that showed that all the treatments and control were not significantly different from each other.

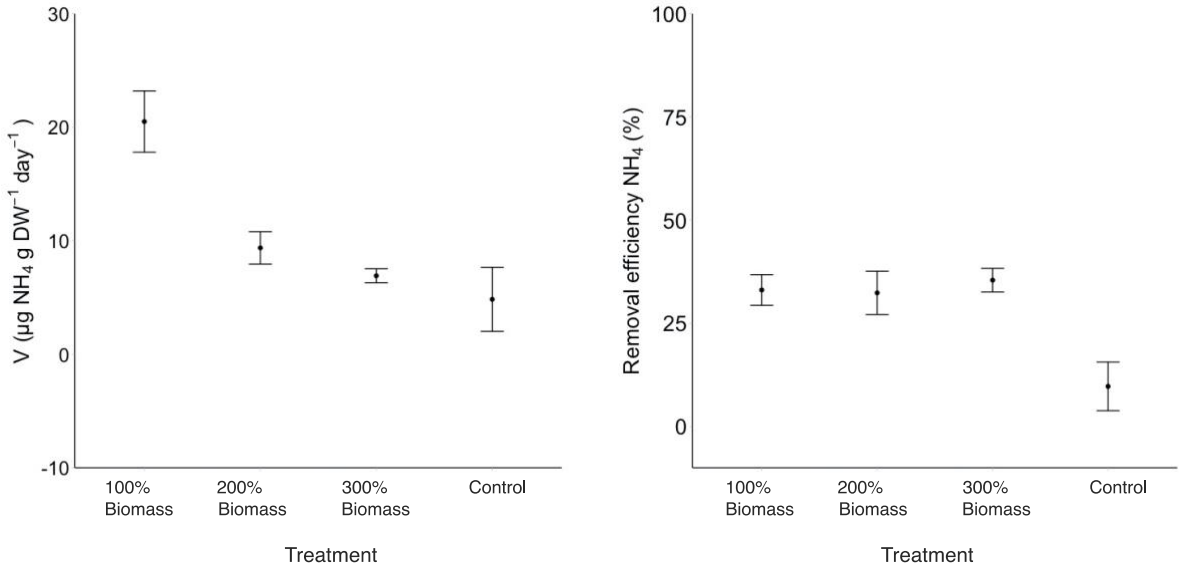

Figure 8. The specific uptake rate (V) and removal efficiency (%) for NH_4^+ in Experiment II at day 7. The biomass treatments were kept in Recycling Aquaculture System (RAS) medium and the control in artificial seawater (with 100% biomass). The error bars in the graphs display the standard error with a significance level of 0.05.

NO_2^-

Generally, the treatments have been leaking NO_{2-} rather than taking up. The leaking was most severe for treatment 100% biomass, which showed a reducing trend for an increasing biomass. The p value of this model was 0.15 and showed to be not significant. Due to combination the low R^2 of the model and the high p value, the results of this model were not further discussed.

NO_3^-

As has been shown in Figure 9, V was significantly different in all treatments, with highest V in treatment 100% biomass and the lowest in control. The difference in uptake was more than 500 µg d DW $^{-1}$. As a result, there was a clear trend visible of decreasing V with increasing biomass. The linear model used showed a very high adjusted R^2 of 0.98 with a p value of $1.189e^{-10}$.

For the removal efficiency, all the RAS treatments showed a removal efficiency between approximately 55% and 80%. The lowest removal efficiency by the RAS treatments was shown in the treatment 100% biomass and the highest in the treatment 200% biomass. The difference between these treatments was 25% in removal efficiency. However, none of the removal efficiencies between the RAS treatments were significantly different from each other. All the RAS removal efficiency did significantly different from the control, which showed a very high

percentage of leaking (-700%). This value has been removed from Figure 9 in order to display the V of the biomass treatments more accurately.

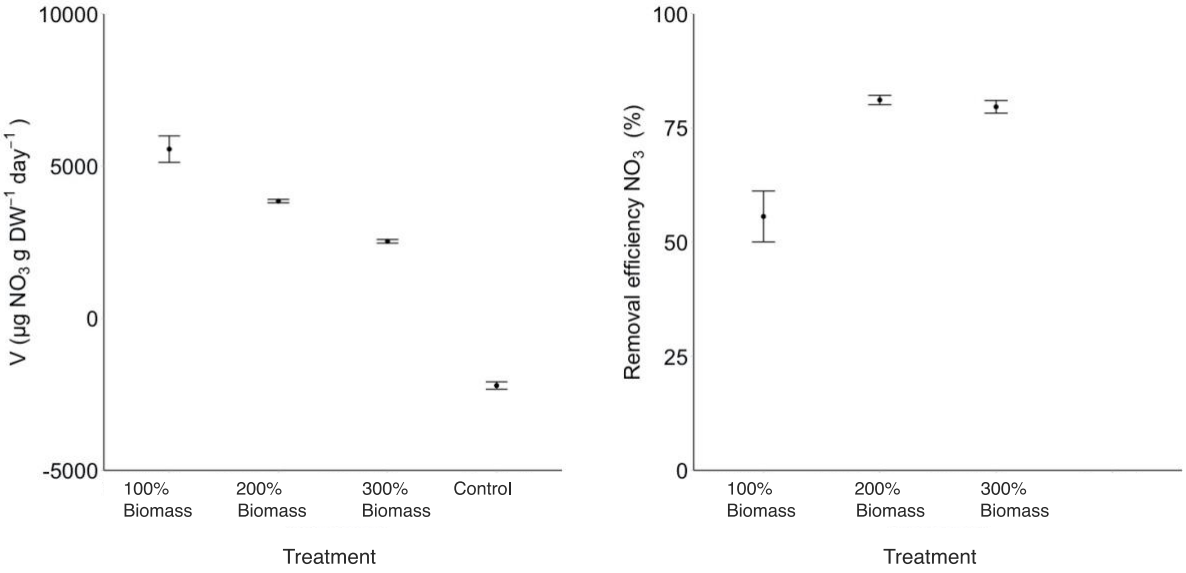

Figure 9. The specific uptake rate (V) and removal efficiency (%) for NO_3^- in Experiment II at day 7. The biomass treatments were kept in Recycling Aquaculture System (RAS) medium. Control value has been removed as this was approximately -700%. The error bars in the graphs display the standard error with a significance level of 0.05.

The nutrient concentrations for NO_3^- were very high in the concentration medium. Unexpectedly, the control also showed very high NO_3^- values. When looking at the NO_3^- values of time, it showed that the nutrient levels in the water only starting to stabilize from day 1 to 3 and showed decrease after day 3. From day 0 to day 1, only the 300% biomass increased treatment decreased in the NO_3^- values. All the other treatments and control showed an increase in NO_3^- values.

PO_4^{3-}

Overall, the values for V for the biomass treatments differed significantly from each other and from control. The highest V of PO_4^{3-} occurs in the treatment 100% biomass and showed a very clear declining trend with increasing biomass. The lowest V was found in the control. The adjusted R^2 was 0.99 with a p value of <0.001.

The removal efficiency of PO_4^{3-} was very high for all the RAS treatments, with the highest occurring in the treatment with 300% biomass. All the RAS treatments showed a removal efficiency between 91 and 99%. These were all not significantly different from each other. All the RAS treatments did significantly differ from the control, showing a removal efficiency of 14%.

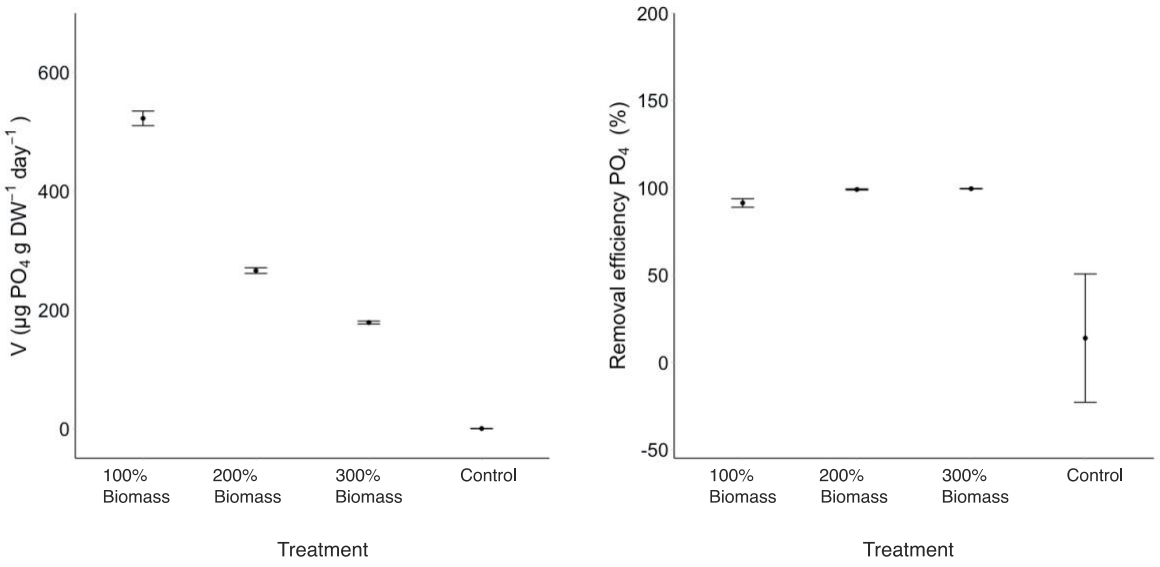

Figure 10. The specific uptake rate (V) and removal efficiency (%) for PO_4^{3-} in Experiment II at day 7. The biomass treatments were kept in Recycling Aquaculture System (RAS) medium and the control in artificial seawater (with 100% biomass). The error bars in the graphs display the standard error with a significance level of 0.05.

The nutrient concentrations in the medium showed that treatment with 100% biomass differed from all the other treatments and control. The value of PO_4^{3-} was highest in the medium for this treatment and lowest for control. When looking at Figure 11, all treatments showed a slight linear trend of decrease in the nutrient concentration over time.

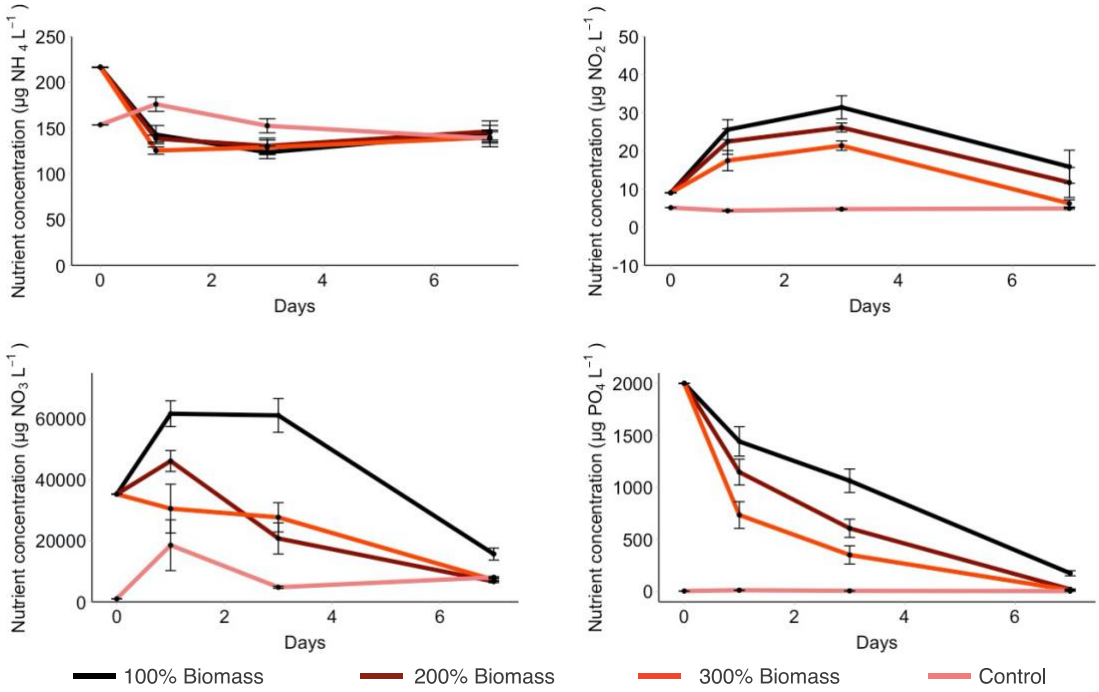

Figure 11. Nutrient concentration in the medium over time (NH_4^+, NO_2^-, NO_3^-, and PO_4^{3-}) by the *Ulva sp.* in experiment II. The legend displays the treatments of 100%, 200%, 300% biomass, and control. The error bars show the standard error with a significance level of 0.05.

3.3 Snail Experiments

The data set contained n=100 samples for the growth experiment. For the grazing experiment the sample size was n=396 for the whole data set. As this experiment was executed twice, on days 1-4 and on days 57-60, the data was plotted with all days included to see if the data between the days showed differences or trends. As the data from day 57 to 60 did not show a stable grazing pattern, it was decided to focus on day 1-4 instead.

Growth Experiment

For the SGR of the snails, all treatments showed an increase compared to treatment 1 with 8.6 °C. The only expection was treatment 10 with 18 °C. The highest increase in SGR was observed between treatment 4 and 8, which have temperatures of 11 and 16 °C degrees, respectively. When looking at size, the SGR of size group 6 mm and of 8 mm were much higher comapred to other sizes and from each other. The remaining size groups of 10 mm, 12 mm and 14 mm did show much difference in SGR. Based on this, there was a clear trend of decreasing growth for increasing size, with the highest SGR for size 6 and the lowest SGR for size 14. All results have been summarized in Table 8 and Figure 12.

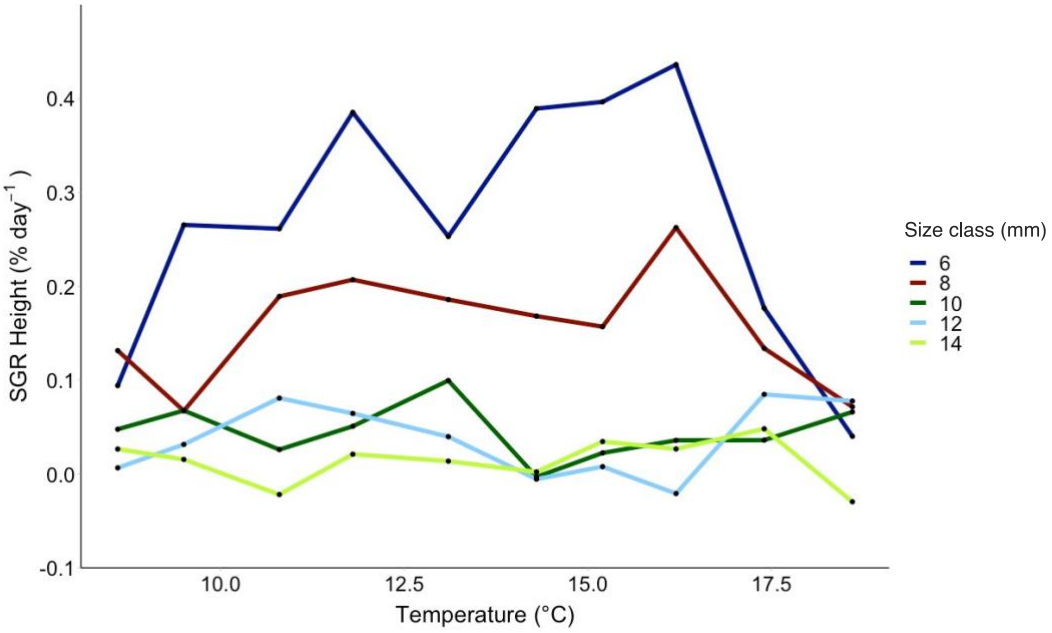

Figure 12. SGR as percental increase in shell height for snails during the experiment. The x-axis shows the different temperature treatments ranging between 8.6 and 18.4 °C.

Table 8. Summary of the SGR as percental increase of shell height.

MEAN SGR (% DAY $^{-1}$)			
Treatment	*Mean SGR (% day $^{-1}$)*	*Treatment*	*Mean SGR (% day $^{-1}$)*
Size 6 mm	0.27	Tr 1: 8.6 °C	0.06
Size 8 mm	0.16	Tr 2: 9.5 °C	0.09
Size 10 mm	0.05	Tr 3: 10.8 °C	0.11
Size 12 mm	0.04	Tr 4:11.8 °C	0.15
Size 14 mm	0.01	Tr 5: 13.1°C	0.12
		Tr 6: 14.3°C	0.11
		Tr 7:15.2 °C	0.13
		Tr 8: 16.2 °C	0.15
		Tr 9: 17.4 °C	0.10
		Tr 10: 18.6 °C	0.04

Grazing experiment

In Figure 13 & Table 9, the grazing patterns per size category and temperature treatment have been summarized. As can be seen, the grazing patterns showed to be stable over time for day 1-4. Additionally, all treatments had a positive effect on the grazing compared to treatment 1 of 8.6 °C, as can be seen in Table 9. The highest grazing activity occurred in treatment 5 (13.1 °C) and 8 (16.2 °C), and the lowest grazing activity in treatment 1 (8.6 °C). The highest grazing was shown by the largest snails of size 12 mm and 14 mm and the lowest grazing was shown by snails of 6 mm. Thus, there was a trend of increasingly increased grazing activity with increasing size.

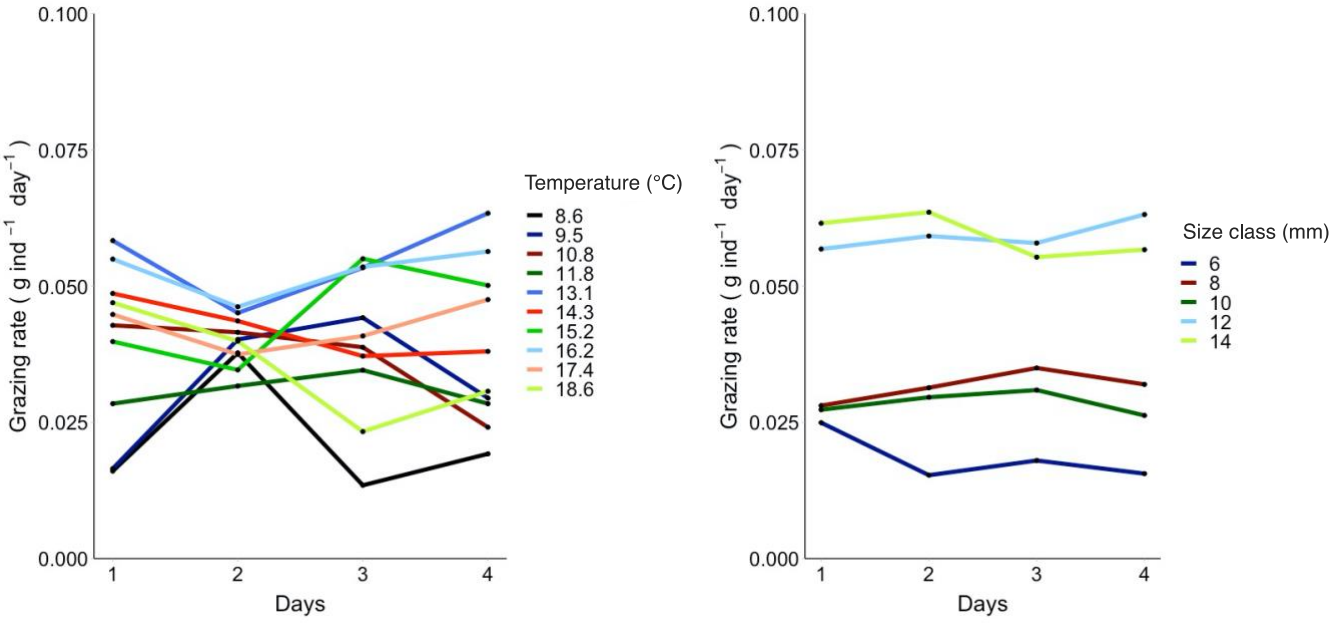

Figure 13. The graphs illustrate the grazing rate (g ind. $^{-1}$ day $^{-1}$) of the snails for days 1-4. Left graph illustrates the temperature treatments 1-10, sorted by temperature associated and the right graphs illustrate the size classes 6 – 14 mm.

Table 9: Mean value for non-standardized grazing rate for the snails

MEAN GRAZING (*G DAY $^{-1}$*)			
Treatment	*Mean g day $^{-1}$*	*Treatment*	*Mean g day $^{-1}$*
Size 6 mm	0.02	Tr 1: 8.6 °C	0.02
Size 8 mm	0.03	Tr 2: 9.5 °C	0.03
Size 10 mm	0.03	Tr 3: 10.8 °C	0.04
Size 12 mm	0.06	Tr 4:11.8 °C	0.03
Size 14 mm	0.06	Tr 5: 13.1°C	0.05
		Tr 6: 14.3°C	0.04
		Tr 7:15.2 °C	0.04
		Tr 8: 16.2 °C	0.05
		Tr 9: 17.4 °C	0.04
		Tr 10: 18.6 °C	0.04

3.4 Model Development

General

As was mentioned in the introduction, the ideal size allocation for the different species was defined by the following parameters: (a) the snails can be harvested at a steady rate, (b) the snails can continuously graze on *Ulva sp.* biomass and (c) over a period of five years, the seaweed biomass does not need to be restocked. Several size allocations scenarios for the different species were run to find the size that meets the requirements above.

Model Input

All parameters that were included have been summarized in Table 10. The robustness scenarios were included and presented as the values in the brackets. Two parameters that are included did not originate from either the snail or Seaweed Experiments. This includes the mortality rate of the snails and the seaweed. These were sourced from literature or chosen as an arbitrary number, after consultation with NTNU and SINTEF, and discussed below.

Table 10. Parameters included in main and additional scenario. n.a. in the table is used to denote a value that is size or time dependent.

PARAMETER	**UNIT**	**BOUNDARY**	**SOURCE**
Time	years	5	Arbitrary number
Harvest size	mm	14	Consultation SINTEF
Size entering co-culture	mm	6	Consultation SINTEF
Size of the farm	m^2	2240	Consultation SINTEF
Density snails	Individuals m^{-2}	156	Consultation SINTEF
Density seaweed	kg FW m^{-2}	1	Ben Ari et al. (2014); Debusk et al. (1986); Neori et al. (1991)
Growth rate seaweed	g day^{-1}	n.a. (±5%)	Seaweed Experiment I
Growth rate snails (size specific)	mm day^{-1}	n.a.	Snail Experiment: Growth
Grazing rate snails (size specific)	g day^{-1}	n.a. (±5%)	Snail Experiment: Grazing
Mortality of L. littorea	%	23 (+5 % -10 %)	Moore, 1937
Mortality of Ulva sp.	%	10 (± 5%)	Arbitrary number

As was mentioned above, several size allocation scenarios were considered to find the scenario that meets the requirements. The following were considered for the size allocation of the total farm size allocation of (snail: seaweed) (a) 1:1 (b) 1:3 (c) 4.5:5.5 (d) 4:6. Below, Table 14, showed how the size allocations affected the space available for snails and seaweed and what the total biomass was for both components in the system. These size allocations were then applied in the explorative model to test how it changed the grazing and harvest of the snails.

Only the in- and outputs of the scenario 4:6 were discussed below, as the requirements of the explorative model were only met in this scenario.

Table 12. Size allocations for snails and the seaweed in the different scenarios.

RATIO	*FARM SIZE*	*SIZE SNAILS (M^2)*	*SIZE SEAWEED (M^2)*	*TOTAL NUMBER OF SNAILS*	*TOTAL KG FW ULVA SP.*
1:1	2240	1120	1120	175000	1120
1:3	2240	560	1680	87500	1680
4.5:5.5	2240	1008	1232	157500	1232
4:6	2240	896	1344	140000	1344

Seaweed Input

The seaweed was stocked with 1 kg FW m^{-2}, as this is found to be the ideal biomass density by the experiments of Ben Ari et al. (2014); Debusk et al. (1986); Neori et al. (1991). The total seaweed input was therefore 1344 kg FW. As the results from the Seaweed Experiment I showed, there was no significant difference between the growth rates of *Ulva sp.* in different RAS dilutions. Therefore, the growth rates of the *Ulva sp.* in the three different RAS treatments have been averaged. To ensure that the explorative model followed the growth patterns shown by the *Ulva sp.* in the RAS over time, the growth rate of the seaweed has been separated in week one, week two, week three and the rest of the year, shown below in Table 11 by day 6, day 15, day 21 and day 27, respectively.

Table 11. Average growth rates of *Ulva sp.* in Experiment I over time intervals

WEEK	**DAY**	**TREATMENT SEAWEED**	**GROWTH RATE**	**AVERAGE GROWTH RATE**
1	6	100% RAS	1.119	1.087
	6	50% RAS	1.069	
	6	25 % RAS	1.075	
2	15	100% RAS	1.027	1.025
	15	50% RAS	1.017	
	15	25 % RAS	1.032	
3	21	100% RAS	1.017	1.018
	21	50% RAS	1.016	
	21	25 % RAS	1.020	
> 3	27	100% RAS	0.996	1.001
	27	50% RAS	1.001	
	27	25 % RAS	1.006	

Snail Input

Growth

Firstly, it has been calculated, the time snails take to reach the next size class using the growth rates using treatment 5: 13.1°C. The results for this have been shown in Table 13. As the growth rate of 6 and 8 showed to be different from each other and the different size classes of snails, the time for these snails to reach the next size class has been calculated separately. It is important to note that this growth cycle did not exactly overlap with the growth cycle used in the explorative model. To ensure that the explorative model can run smoothly, and all the size groups of the snails spend similar time in the system, the days until the snails reach the next size class were slightly smoothed over (See Table 13). After alteration, it took both size group 6 and 8 157 days to reach the next size class, and size 10 needs 313 days to reach size group 14. As this explorative model was run for 5 years, the cages were restocked for the snails after year 4 to ensure that at approximately the end of year 5 all the cages were empty.

To ensure that harvesting could take place easily, the snails were sorted into four "enclosures" according to the different sizes of 6 mm, 8 mm, 10mm and 12 mm. Assuming that the four enclosures hold the same number of snails of the total 140 000 snails in the system, every enclosure contained 35 0000 snails. Hence, every 157 days, when the snails moved cages, 35 000 snails of 6 mm entered the system (see Table 15).

Table 13. Growth rates and time needed to reach the next snails per size class. Growth rates are based on treatment 5 (13.1 °C)

ENCLOSURE	*SNAILS PER ENCLOSURE*	*DAILY GROWTH RATE*	*SIZE (MM)*	*DESIRED SIZE*	*TIME TO REACH NEXT CLASS (D)*	*ASSUMPTION FOR MODEL (D)*
1	35 000	1.002	6	8	151	157
2	35 000	1.001	8	10	176	157
3	35 000	1.001	10	12	163	157
4	35 000	1.001	12	14	137	157
TOTAL	**140 000**				**627**	**627**

Grazing

To calculate the total grazing per year, the data from snail grazing day 1-4 in treatment 5 has been applied. All data has been summarized in Table 14. Grazing has been calculated per size in the system and showed that the larger snails consumed more biomass. Daily, more than 4 kg FW was consumed by all the snails in the systems. Over the course of one year, almost 1700 kg FW was consumed. As the grazing stable has been to be stable in the Snail Experiment, the grazing in this model was also assumed to be stable.

Table 14. Grazing per size class per day and per year summarized per size allocation scenario.

SIZE ALLOCATION 4:6					
Size class present	*Mortality rate*	*Snails per size class (after mortality)*	*Grazing (g ind^{-1} day $^{-1}$)*	*Grazing (kg per day)*	*Grazing (kg per year)*
6	0.23	26950	0.019	0.50	182.13
8	0.23	26950	0.033	0.88	322.66
10	0.23	26950	0.017	0.45	165.31
12	0.23	26950	0.105	2.82	1028.42
Total		**107 800**		**4.65**	**1698.52**

Mortality

The mortality for the snails was based on a paper by Moore (1937) that measures the mortality of *L. littorea* in several natural habitats. The lowest mortality rate that was found was 23% and was measured in Yealm (UK) on a stony habitat for snails that showed a stable growth rate. As there was no value available for the mortality rate of *L. littorea* or other similar gastropods that were cultivated, it was decided to use this value. As it was assumed that this value overestimates the mortality, as there was no natural predation in aquaculture facilities, the robustness scenario considered a decrease of 10% in mortality and an increase of 5%.

Lastly, it was not possible to find a specific value for the mortality rate for *Ulva sp.* in aquaculture systems in literature. Therefore, it was assumed that the mortality on average would be 10%. This value has been consulted with SINTEF and NTNU.

Explorative Model Output

Below, the following results were presented for size allocation scenario of 4:6: (1) total input and output of the system of snails and (2) seaweed consumption by the snails in Table 15 and 16, respectively.

Every 157 days, 35 000 new snails were supplied into the system and every 157 days 26 950 snails can be harvested, when applying the mortality rate of 23%. As the system was fully stocked on day 0 with the total number of snails present in classes 6, 8, 10 and 12, these snails were harvested on day 157, 313, 470 and 627, respectively. Hence, the input of snails was highest on day 0. Additionally, the output on the aforementioned days was higher as well. The day count for input and output on the five-year basis has been shown in Table 15.

Table 16 illustrated the total seaweed consumption by the snails in the system on the five-year basis. The table lay-out that includes in total 4 rows for year 1 ensured that the growth pattern on the *Ulva sp*. in RAS systems was mimicked. Table 16 illustrates that after five years there was still a seaweed surplus of 65 kg FW. Thus, all the requirements of the explorative model were met that the snails can continuously be harvested during the five years, the snails can continuously graze on the seaweed biomass and the seaweed biomass only has to be stocked once.

Table 15. In- and output for the snails and seaweed for the size allocation of 4:6

SIZE ALLOCATION 4:6				
YEAR	*In- and output days*	*Size increase in system (mm)*	*Input Snails*	*Output Snails*
1	0-157	12-14	35 000	26 950
1	0-313	10-14	35 000	26 950
2	0-470	8-14	35 000	26 950
2	0-627	6-14	35 000	26 950
3	157-784	6-14	35 000	26 950
3	313-940	6-14	35 000	26 950
4	470-1097	6-14	35 000	26 950
4	627-1254	6-14	35 000	26 950
4	784-1411	6-14	35 000	26 950
5	940-1567	6-14	35 000	26 950
5	1097-1724	6-14	35 000	26 950
5 + 56 DAYS	1254-1881	6-14	35 000	26 950
	TOTAL		420 000	323 400

Table 16. The net growth of seaweed for the size allocation of 4:6 over a period of five years.

SIZE ALLOCATION 4:6							
YEAR	*Day*	*Start biomass (kg FW)*	*Growth rate*	*Grazing rate snails (kg t_i^{-1})*	*Total biomass (kg FW)*	*Net biomass (kg FW)*	*Surplus*
1	7	1344	1.087	32.57	2416.20		
1	7		1.025	32.57	2877.11		
1	7		1.018	32.57	3254.64		
1	344		1.001	1600.80	5121.34	4609.20	2910.68
2	365		1.001	1698.52	4708.63	4237.76	2539.24
3	365		1.001	1698.52	4107.75	3696.97	1998.46
4	365		1.001	1698.52	3232.91	2909.62	1211.10
5	365		1.001	1698.52	1959.20	1763.28	64.76

Robustness Scenarios: Main scenario

In total, 8 different robustness scenarios were included for the 4:6 size allocation scenario. The exact effects of these scenarios can be found in Table E.2 and E.3. The effects have been shown in tables of seaweed consumption and total in and output.

Overall, the input of seaweed was most sensitive to most changes that were tested in the robustness scenarios. These scenarios included changes in grazing rates, growth rates of the seaweed and mortality rates of the snails. For most scenarios, this meant that the input of seaweed biomass doubled over the five-year period as there was a need for the restocking of biomass. Consequently, this increased the output of seaweed as well in most scenarios. The exception was the +5% of mortality in seaweed, which increased the input of seaweed but decreased the output of seaweed. Scenarios that reduced the seaweed grazing or increase the seaweed biomass, maintained the input of 1344 kg FW, and increased the output as well between approximately 1000 to 3000 kg FW. The in- and output of the snails was only affected by the changes in mortality rates in the snails. The changes in in- and output have been shown in Figure 14 and 15.

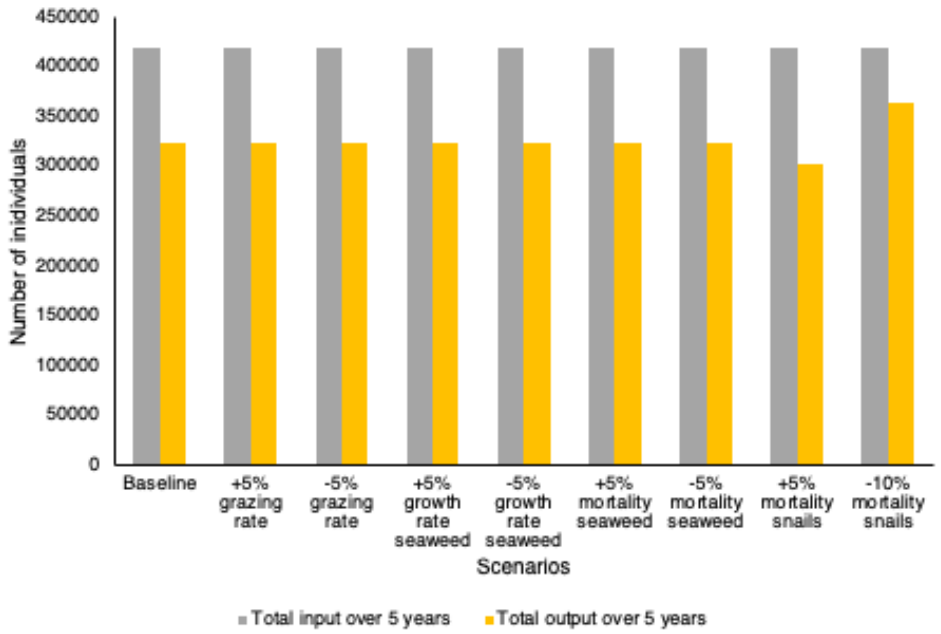

Figure 14. in- and output of baseline scenario (4:6 size allocation) for the snails.

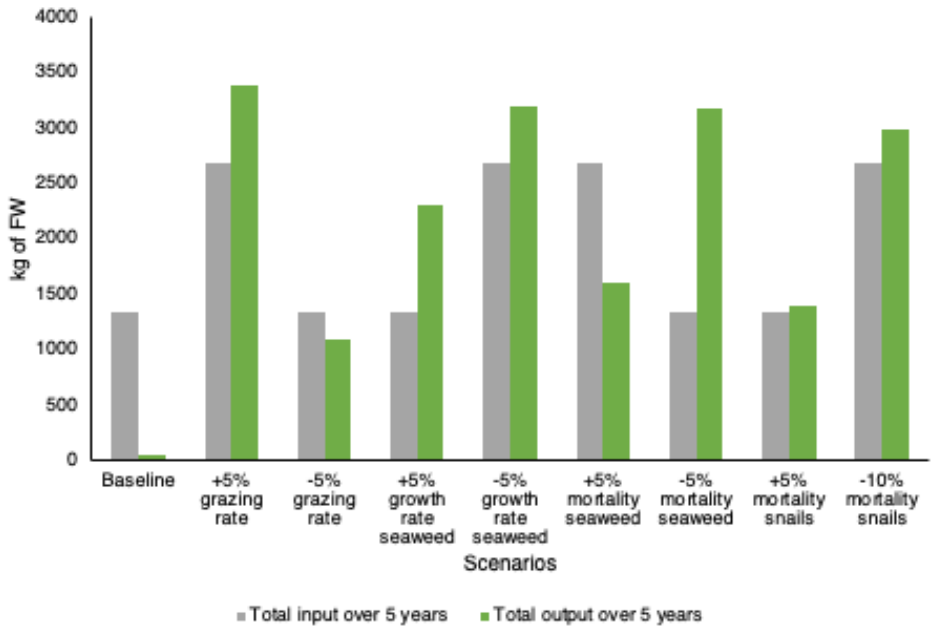

Figure 15. in- and output of baseline scenario (4:6 size allocation) for the seaweed.

4. Discussion

The results of this study showed that *Ulva sp.* can successfully be cultivated in RAS wastewater with high NO_3^- levels. They showed that the SGR and chemical composition were significantly affected by the RAS wastewater in comparison to the control with the artificial seawater. Both the growth rate and the chemical composition showed on average an increase of approximately 300%. Additionally, the successful uptake of NO_3^- by *Ulva sp.* in the RAS wastewater showed that utilization of this species is not limited to the bioremediation of NH_4^+ products only. To translate this potential of this species into a tangible benefit to the RAS producers, an explorative model simulating the co-culture of an *Ulva sp.* and macroalgivore grazer in RAS wastewater was developed. This explorative model illustrated that a biomass of 1344 kg FW that was cultivated in RAS wastewater was sufficient to continuously supply 420 000 snails with *Ulva sp.* biomass to graze on and more than 300 000 snails to be harvested over a five-year period.

4.1 Seaweed Experiments in Context

Growth

The hypotheses that this experiment were based on were the following: (1) the growth of the *Ulva sp.* is significantly altered by being grown in a RAS medium and (2) the concentration of RAS water influences the changes in growth rate. The first hypothesis was supported by the results that have shown that the growth rate between the RAS treatments and the control was significantly different over time and that *Ulva sp.* being cultivated in RAS medium had a positive effect on the SGR of the seaweed.

The results of Seaweed Experiment I did not support the second hypothesis regarding the effect of the concentration of RAS water. In almost all results, it was shown that the different concentrations of RAS did not significantly change the growth or chemical composition. This may be caused by the fact that for some nutrients, there were no significant differences between the composition of the water for the RAS treatments. The lack of differences between treatments and control could have resulted from the Red Sea Salt that was used to increase the salinity of the RAS water.

Despite of the lack of significant difference between the RAS treatments, the *Ulva sp.* still showed interesting trends in regard to the variation in growth between these treatments. Firstly, it seemed that the seaweed reacts differently to the different RAS treatments. The lower RAS concentrations showed a later peak of growth compared to the 100% RAS treatments. For the

RAS treatments of 25 and 50%, the growth spike occurred on day 9, compared to day 6 of 100% RAS. In addition, the 100% RAS treatments also showed the sharpest decline in SGR over time and displayed a negative growth at the end of the experiment. The lower RAS concentration treatments showed a more stable growth pattern and at the end of the experiment both had a higher SGR than 100% RAS.

Several studies report the growth of *Ulva sp.* cultivated in both RAS and other nutrient rich media. Ale et al. (2011) enriched the growth medium with 350 µg L^{-1} for both $NaNO_3$ and NH_4Cl. This value for NH_4^+ was higher than in this study, consisting of 216 µg L^{-1} and lower for NO_3^-, consisting of 35 283 µg L^{-1}. They found a maximum SGR of 16.4 day^{-1} which was higher than in this experiment where the values were between a maximum SGR of 10 and 11 % day^{-1}. Additionally, the SGR found by Ben Ari et al (2014) on average was 13.3, which was also higher than in this study. The NH_4^+ levels in the study conducted by Ben Ari et al. (2014) were almost 1000 times higher than the values in this experiment.

The higher growth rate in the experiments could be explained by the higher concentration of NH_4^+, which is the preferred product for uptake by the seaweed (Luo et al. 2012). Additionally, Steffensen (1976) found that the optimal levels of NO_3^- for *Ulva sp.* is 0.6 g m^{-3} and that increasing NO_3^- above these levels can reduce growth. The nutrient levels received from the RAS water for these experiments varied between 35 and 8.75 g m^{-3} for the different RAS treatments. As this was far above what Steffensen (1976) found, it could explain the stagnating growth of *Ulva sp.* over time and the lower values compared to literature. As the 25% RAS was relatively closest to the optimal level, it could explain why this RAS treatment was more stable and showed a higher SGR overall.

Tissue

As was shown, the chemical composition was significantly altered by the RAS wastewater. For all results, the control was significantly different from the RAS treatments. Additionally, it was shown that for all RAS treatments the C:N and N:P ratio was lower and the protein content and the organic phosphate concentration were higher, compared to the control. This supported the hypothesis that (3) the chemical composition of the *Ulva sp.* was significantly altered by being grown in a RAS medium. Like to the SGR results, the results of this study did not support the hypothesis (2) that the concentration of RAS water influences the changes in growth rate and chemical composition.

All the tissue parameters included in the Seaweed Experiment I, showed the strongest change in the first week of the experiment compared to the remaining three weeks. It showed that keeping the *Ulva sp.* biomass in RAS wastewater for one week was enough to raise protein concentrations that are interesting for using the biomass as a feed source. Similar to the growth experiments, the highest values for proteins were found in the lowest RAS treatment of 25% at the end of the experiment. Again, this suggests that the concentration of 25% RAS water created optimal performance for *Ulva sp.*.

The protein content is important to determine the suitability of the macroalgae as gastropod feed and Shuuluka et al. (2013) stated that protein is the most critical component for determining the nutritional value of food. Several studies found a high protein content for seaweed cultivated in fishpond effluent. Myusa & Neori (2008) found a protein value ranging between 17 & 44% for *Ulva sp.* cultivated on fishpond effluent with varying N content. The higher protein content found by Myusa & Neori (2008) could be explained by the use of the conversion factor of 6.25. Protein values are often overestimated when using this factor as some of the nitrogen is not used for proteins in seaweed tissue (Angell et al., 2016; Biancarosa et al., 2017). Therefore, this study used a conversion factor specifically for green algae of approximately 4.3, which explains the lower findings. Additionally, Myusa & Neori (2008) found that found increasing the nitrogen content in the fish effluent, increases the protein content significantly. This is in contrast with the results from this study, that showed no difference between the treatments. This could have been caused by the lack of significant differences between the treatments for the nitrogenous products.

The protein value at the start of the experiment was around 12%. This was on the lower side for crude protein value of green algae as Fleurence (1999) showed that the natural range of crude proteins for green algae is between 10 and 26%. This is supported by Angell et al. (2016) and Biancarosa et al. (2017) who showed that wild harvested green algae have a protein value between 15% and 22 %. However, the protein values of the *Ulva sp.* before the experiment do correspond with the values found by Shuuluka et al. (2013) for wild *Ulva sp.* species, showing a maximum of 11%. As the null value of the seaweed used in this experiment was significantly lower than what is commonly found for wild harvested *Ulva sp.*, this experiment successfully proved that the cultivation of *Ulva sp.* in RAS wastewater increases protein levels and so the suitability for potential uses, such as macro-algivore feed.

The increase in nitrogen (N) and phosphorous (P) products richly available positively affected all tissue contents, with the exception the carbon (C) content. For the C content, there were almost no significant differences among all RAS treatments and with the control at the end of the experiment. This could be explained by the fact that the both the RAS water and the artificial water contained the Red Sea Salt to increase the salinity, which may have contained high values of C.

The nutrient ratios between C:N and N:P in the RAS treatments all showed to be significantly different to the artificial seawater. These nutrient ratios are important to consider when looking at nutrient limitations and as an index for physiological status of algae (Gómex Pinchetti et al., 1998). Lubsch & Timmermans (2018) found that *Ulva sp.* is twice as likely to suffer from N-limitation than P-limitation when considering the Redfield ratio of 16:1 for N:P. By cultivating the *Ulva sp.* in the nitrogen rich medium RAS wastewater, this limitation can be successfully combated. A C:N ratio of 10:1 is considered to be optimal for *Ulva spp.* (Tonk & Jansen, 2019). Therefore, the *Ulva sp.* RAS treatments at the end experiment showed a nearly optimal C:N ratio of 7:1. This was in strong contrast with the C:N ratio of the control at the end of the experiment. The C:N ratio of the control was approximately 23:1. In addition, the RAS wastewater medium also optimized the N:P ratio of the *Ulva sp.* The optimal N:P ratio for *Ulva lactuca* was estimated to be 30:1(Lubsch & Timmermans 2018). In this experiment, the N:P ratio for the RAS treatments was 28:1.

It is important to note that none of the RAS treatments and control were nutrient limited, as all values were above the threshold from Fujita (1985) of 0.022 μmol NH_4^+ L^{-1}, 0.003 μmol NO_3^- L^{-1} and 0.008 μmol PO_4^{3-} L^{-1} (Lubsch & Timmermans, 2018). It is therefore difficult to explain why the growth and tissue content concentrations of controls displayed trends similar to starved algae. Gómez Pinchetti et al. (1998) found that after a week in nutrient limited medium, growth decreased and stagnated, similar to trends shown by the algae in control. Furthermore, Floreto et al. (1996) found that after 4 days of *Ulva sp.* biomass in medium with starved nutrient levels showed negative specific growth rate and loss of biomass. This occurred as well with *Ulva sp.* in the control at the end of the experiment. In addition, the increase in C:N ratio in the control shows similar trends as N starved algae as well. When nitrogen limits growth, carbohydrate synthesis dominates and is responsible for the rise in polysaccharide levels and so an increased C:N ratio (Gómez Pinchetto et al., 1998). It remains difficult to comment on why the algae show this behavior as the nutrient levels were above starvation. It therefore may show that Red Sea salt was not sufficient to sustain algae growth.

Uptake

Additionally, the results illustrated that *Ulva sp.* successfully takes up nutrients from the RAS waste stream and for almost all nutrients showed a significantly increased specific uptake rate and high removal efficiency, compared to *Ulva sp.* in the control. The higher specific uptake rate and removal efficiency of NO_3^- compared to NH_4^+ shown by the *Ulva sp*. indicated that this species can successfully take up and assimilate important waste products from RAS water. In addition, this experiment showed that biomass density affects specific uptake but not the removal efficiency. The hypotheses that concerned the Seaweed Experiment II included (4) that *Ulva sp.* will take up the nutrients from the RAS waste stream and (5) that the uptake rate and removal efficiency is influenced by the biomass density. Overall, the 100 % biomass treatment showed the highest specific uptake rate for most of the nutrients. In contrast, the highest removal efficiency was usually attained by the 300% biomass treatment. However, it must be noted the differences among biomass treatments regarding removal efficiency were not significantly different for most nutrients. These results suggested that the seaweed was most efficient per g DW in the lower biomass gradients and that these lower biomass treatments could, therefore, reach similar removal efficiencies as higher biomass gradients. Therefore, these results supported the fifth hypothesis that stated that the specific uptake rate was influenced by the biomass density. In contrast, the removal efficiency was not significantly affected by the biomass density.

The highest removal efficiency was found for PO_4^{3-}, whereas the highest specific uptake rate was found for NO_3^-. The highest removal uptake rate for PO_4^{3-} may be explained by the high presence of NO_3^-, which showed to be taken up more easily when there are high levels of NO_3^- present (Shahar et al., 2020). Both the removal efficiency and the specific uptake rate were lower for NH_4^+, which was unexpected, as the uptake of this product requires less energy (Shpigel et al., 2019).

These results were also in contrast with previously conducted experiments that looked at the bioremediation potential of *Ulva sp*. in fishpond effluents. Both Ben Ari et al. (2014) and Myusa et al. (2006) found a higher uptake rate of NH_4^+ m^{-2} d^{-1} than in this experiment. After recalculating the specific uptake rate to the unit of their use, the uptake of g NH_4^+ m^{-2} d^{-1} was a fraction of their values Ben Ari et al. (2014) found an uptake of 1.44 to 1.8 g NH_4^+ m^{-2} d^{-1}, whereas Myusa et al. (2006) showed a higher uptake of TAN of 6.5 g NH_4^+ m^{-2} d^{-1} for *Ulva lactuca*. In the uptake experiment in this study, the *Ulva sp.* in this study showed an uptake 0.005 g NH_4^+ m^{-2} $d^{-}1$. The low uptake of NH_4^+ in this study could be explained by the high

values of NO_3^-. Uptake of NH_4^+ may be inhibited when there are multiple sources of N available or there is a high availability of NO_3^- (Li et al., 2019). Fan et al. (2014) found that when the ratio between NO_3^- / NH_4^+ was lower than 2.2, *Ulva sp.* preferred NH_4^+. However, in higher ratios, the uptake of NO_3^- was favored over NH_4^+ (Fan et al., 2014). The ratio between NO_3^- / NH_4^+ in this experiment was approximately 160 and therefore well over the value in which *Ulva sp.* prefers NH_4^+. The uptake rate of NO_3^- in this experiment support the preference for NO_3^- mentioned above. In contrast with other bioremediation experiments with high NH_4^+. values, the uptake of NO_3^- was much higher in this experiment. Both Ben Ari et al. (2014) and Wang et al. (2007) reported an uptake for NO_x between 0.06-0.45 g NO_x m^{-2} d^{-1} for *Ulva sp.* The results of this study were much higher, reaching 12 g NO_3^- m^{-2} d^{-1}.
It is important to note that the of NO_3^- was not immediate in this experiment. The nutrient levels only showed to decrease after day 3. Naldi & Wheeler (2002) mention that there is a lag period before the *Ulva sp.* start using the NO_3^- due to a metabolism shift. This may explain that the nutrient levels for NO_3^- only start to decrease after day 3.

When comparing the results of this study to the literature, it became clear that the uptake of nutrients by *Ulva sp.* can vary significantly between studies and is dependent on the medium it is kept in. Even though *Ulva sp.* may prefer NH_4^+, it still shows to be successful at removing NO_3^-. These comparisons show the wide range of capabilities of *Ulva sp.* for bioremediation, by being able to adapt to the medium its kept in, including RAS wastewater.

4.2 Snail Experiments in context

Growth

None of the hypotheses regarding the snail growth and grazing could be supported due to the lack of statical confirmation. However, the results did indicate that both growth and grazing were dependent on size rather than temperature. The results of the snail growth experiment showed that there were no trends visible among the temperature treatments. This was not in line with the hypothesis of this study, as it was assumed that temperature increasingly increases growth, due to their poikilothermic status (Dehnel, 1995; Frick et al., 2018). This may be explained by the findings of Hoefnagel & Verberk (2017) that found that growth performance is more affected by parameters during rearing than by "acute" parameters during the experiments. Additionally, they show that variation in the data is more related to individual differences in rates of food and oxygen consumption (Hoefnagel & Verberk, 2017).

Unfortunately, there is not much data available on the growth rates of *L. littorea* cultivation systems. Growth rates of wild populations of L. littorea show to vary per location and per age group (Moore 1937; Robson & Williams, 1971; Williams, 1964). The data found by the authors aforementioned, found that the highest growth rates are attained in the youngest snails and over a period of 4 years reduced from 0.027 to 0.005 mm day $^{-1}$. This supports the strong effect of increasing size and decreasing growth illustrated by this experiment. Furthermore, Cashmore & Burton (1998) found that over a period of 21 days, snails being fed on *Ulva sp.* grew approximately 0.35 mm in shell height (0.017 mm day^{-1}). These growth rates are very similar to the ones found in this experiment for the snail sizes 6 and 8 mm, 0.018 and 0.014, respectively. As the goal of on-growing would be to realize rapid growth rates that are higher than in nature (Cummins et al., 2002), the results of this study show that there is still room for improvement to optimize the grow-out period.

Grazing Behavior

As mentioned above, periwinkles show the fastest growth when feeding on *Ulva sp.* biomass (Cashmore & Burton, 1998). Therefore, monitoring the grazing behavior is important to understand the dynamics and to optimize how much *Ulva sp.* is needed when periwinkles are ongrown in aquaculture systems. Unfortunately, there are not many sources that report the exact grazing of this species. Wilhelmsen & Reise (1994) report grazing rate of 0.11 g day $^{-1}$ of periwinkels on *Enteromorpha*, another green algae. This value is more than double the value found by this research. However, Wilhelmsen & Reise (1994) mention that the grazing on *Ulva sp.* can be three times lower. When taking this remark into consideration, the grazing could be close to what was found in this research. This shows that grazing that was exhibited by the snails in this experiment is similar to values found in literature. Therefore, the grazing rates found in this experiment reflect the grazing behavior, which strengthening the robustness of the explorative model.

4.3 Potential of Co-Cultivation & Improving Management Practices

By combining the data, an explorative model for the co-culture of *Ulva sp.* and *L. littorea* was developed. Overall, the results of the experiment executed suggested a potential for the co-culture of *Ulva sp.* and *L. littorea* in the RAS waste stream. The growth of *Ulva sp.* was strengthened by being in the RAS medium, whilst significantly increasing protein levels. However, the instability, shown as the decline in SGR for the 100% RAS treatment over time

illustrated that this treatment may contain too high NO_3^- concentrations. This suggestion is supported by the findings of Steffensen (1976) that show that all treatments contain NO_3^- values over the optimum. However, the results from Seaweed Experiment I in regard to growth and chemical composition, show that the 25% RAS treatment attains the most desirable results when using the *Ulva sp.* biomass as a macro-algivore feed resource over a longer period of time. Therefore, output of the farm can be improved by diluting the RAS water to desired nutrient concentrations of 25% RAS water or lower.

Additionally, *Ulva sp.* shows that it can successfully take up NO_3^-, an important waste product from RAS water. The results from the long-term uptake experiment additionally show there does not seem to be a need to increase the biomass to reach higher removal values, as there mostly is a lack of significant differences among the biomass treatments.

The snails show that ongrowing in aquaculture systems did not decrease growth compared to behavior in natural habitats and that the grazing patterns were stable. By supplying the system with 1344 kg FW, more than 400 000 snails can graze the biomass continuously and more than 300 000 snails were harvested over the period of five years.

However, it seems that there is opportunity to further optimize the outputs of the explorative model. The model currently uses a stocking density of 1 kg m^{-2} for *Ulva sp.*. However, Vandermeulen & Gordin (1990) found that stocking density can be increased up to 4 kg FW m^{-2} without reducing growth. By increasing the stocking density of the seaweed biomass, the output can be significantly increased. This offers the producer additional valuable biomass.

It can also be considered to increase the stocking density of the snails. However, it should be noted that in natural populations, growth can be depressed when density is increased (Cummins et al., 2002; Petraitis, 2002). A cause of this decrease in growth is partly caused by increased competition for resources. As this should not be an issue in cultivation systems, it should be researched how increasing the stocking density of snails influences the growth.

4.4 Limitations & Challenges

Limitations in Study Design

There are several challenges and limitations to the use of the data gathered in these experiments and in the explorative model. Firstly, the growth measured in Seaweed Experiment I spanned only over 27 days and, therefore, may not have reflected the growth of *Ulva sp.*in RAS wastewater over a longer period. On day 27, several flasks showed a decreasing biomass, with

the loss of pigments and an overall loss of firmness in the thalli. Knowing the long-term growth of *Ulva sp.* biomass in RAS medium could significantly impact the input and output of *Ulva sp.* for the co-culture model. The growth and chemical results from Seaweed Experiment I showed trends that 25% RAS water may be optimal compared to 100% RAS. However, as none of the nutrient levels between the RAS treatments were significantly different from each other for nitrogenous waste products, these results should be used with caution.

A further limitation was caused by the controls in Seaweed Experiment I, that showed signs of nutrient limitations. However, the nutrient levels were above the threshold set for starvation. It could be that the Red Sea salt used to increase the salinity of the water did not create suitable conditions for the algae to grow. Additionally, as was mentioned in the methodology, there were no null samples taken from the artificial seawater, only on day 1. Therefore, it remains difficult to fully understand the reaction of the algae to the control cultivation medium. The trends that the algae showed may also not have reflected algae in natural habitats. The goal was not to compare *Ulva sp.* in RAS waste stream with starved algae but with *Ulva sp* with 'natural' growth conditions. Therefore, it remains difficult to comment on the comparison between *Ulva sp.* in RAS waste stream and *Ulva sp.* under more 'natural' conditions.

Furthermore, the study design of the Snail Experiment by SINTEF Ocean, did not allow for any statistical testing, as there was an absence of true replicates. Therefore, this study could not provide any conclusions about the effects of temperature and size on grazing and growth of the snails. Consequently, any trends mentioned in this research should be treated with caution. Additionally, for the development of the co-culture model the size specific grazing and growth rates are used from treatment 5. This means that there was only one replicate available for the value used and may not reflect general behavior of the specific snail sizes in this treatment. Cashmore & Burton (1998) found that there were large variations per individual growth rate, and this may well be the same in grazing rate. The use of these values may therefore not truly reflect the general behavior of snails of that size. In addition, the snails' experiment was executed during spawning season and therefore does not reflect maximum growth rate.

Challenges in Application of the Explorative Model

The limitations in the Seaweed Experiment I and II and the snails research impact the accuracy of the explorative model. Therefore, the output of the explorative model should be used with

caution for both snails and seaweed. There are several values that are used in the explorative model that may not reflect realistic cultivation scenarios. First, the mortality of snails may be overestimated, as the only value for mortality could be found was in natural habitats. A drop of mortality could significantly influence the input of seaweed and the output of snail. This has been partly mitigated by running a robustness scenario that takes into account a 10% drop in the mortality. However, to improve the accuracy of the explorative model, more attention should be paid to measuring this value in cultivation systems with *L. littorea.*

Secondly, variation in growth values reported in literature show that *Ulva sp.* is difficult to cultivate and is substantiated by the fact that both during pilot study and towards the end of this study, the seaweed showed disintegration and was unstable. The explorative model assumes that the growth stabilizes, and therefore, uses the growth data of the seaweed on day 27. However, if the growth rate continuous to decline and ultimately reduces biomass, it could change both the input and output of the seaweed biomass significantly. To increase the accuracy of the explorative model and ensure that the snails can graze continuously, more research should be devoted to studying the long-term growth patterns in a NO_3^- rich medium and finding the right parameters for *Ulva sp.* cultivation.

4.5 Future Work & Prospects

The above-mentioned limitations of this work highlight the potential for improvement. Firstly, the research shows that more attention should be paid to fully understanding the growth patterns of *Ulva sp.* biomass in RAS wastewater. This will aid in the robustness of the explorative model and enhance the output of biomass to the producer. It should further be examined what the potential is of increasing the stocking density from 1 to 4 kg m^{-2}. Aspects that should be considered are mortality, altered growth rate and chemical composition.

Secondly, to optimize explorative model output, more research should focus on finding the right parameters that can increase the growth rate of the snails. Currently, the snails harvested from the system at 14 mm fall within the category “small” by Cashmore & Burton (1998). To fetch higher prices and increase the output, finding the right parameters to optimize growth should be given sufficient attention. To increase the accuracy of the output of the explorative model, acquiring knowledge on this is crucial.

Thirdly, a feasibility study should be conducted to test if the explorative model can be applied in real life. Specific focus should be on the economic and logistic feasibility. Important aspects here are researching if the wild stocks of *L. littorea* and *Ulva sp.* are sufficient to supply the input, the economic costs and benefits of the operation and researching the market to see if there is sufficient demand of both seaweed and snails.

5. Conclusion

The Norwegian aquaculture sector and government share a vision to increase the aquaculture production considerably. Practices such as Integrated Multi-Trophic Aquaculture and Recirculating Aquaculture Systems offer opportunities in waste management for the nutrient fluxes that could result from the increased production. Integrating lower trophic species, such as seaweeds, offers a remediation potential for the high nutrient flux of the waste products and simultaneously offers the producer an additional valuable biomass. The bioremediation and cultivation potential of seaweeds such as *Ulva spp.* in RAS waste streams has been challenged by the high nitrate (NO_3^-) concentrations. This thesis aided in the exploration of this potential co-culture by examining the prospective growth of *Ulva sp.* in RAS wastewater and the effects on growth and chemical composition. Here, the effects of RAS varying concentrations in the cultivation medium were tested. In addition, this thesis explored the uptake behavior by *Ulva sp.* of, among other nutrients, NO_3^- that is the most important compound from RAS wastewater. The influence of biomass density on uptake and removal efficiency was tested in this experiment as well.

This study illustrated that *Ulva sp.* can successfully be cultivated in the wastewater of a RAS facility. The *Ulva sp.* biomass showed enhanced growth and chemical composition, compared to *Ulva sp.* cultivated in artificial seawater. On average, the specific growth rate and protein value are 337 and 319% higher, respectively. However, this difference was lowered to 83% for the specific growth rate for the *Ulva sp*. at the end of the experiment. This was mostly influenced by the sharp decline in SGR shown by the 100% RAS treatment. When only considering the 25% RAS concentrations at the end of the experiment, the specific growth rate was still 350% higher. For all RAS concentrations, both the C:N and N:P ratios in the tissue show to be around optimal levels of approximately 1:9 and 1:28, respectively. Again, most desirable results, as in highest protein content and lowest C:N and N:P ratios, were attained in the 25% RAS treatment.

The combination of these insights was then used as input into an explorative co-cultivation model, that examines the in- and outputs of *Ulva sp.* and *L. littorea* biomass produced in RAS wastewater over a five-year period. Over a five-year period, using an *Ulva sp.* biomass of 1344 kg FW, more than 300 000 snails could be harvested. The explorative model shows that the snails can continuously graze on the seaweed biomass and that over a five-year period can be

harvested at a steady rate. Robustness scenarios show that reducing both mortality of *Ulva sp.* biomass an*d L. littorea* offer most opportunities to increase output.
Moreover, the uptake experiments show that *Ulva sp.* can successfully take up the compounds from the RAS waste stream. It was found that in most cases increasing the biomass negatively affects the specific uptake rate. The removal efficiency showed that whilst there are trends visible with increasing biomass and increasing removal efficiencies, most of these differences are non-significant. For nitrate, *Ulva sp.* can attain removal efficiencies between 55- 80%, with a specific uptake rate between 5600 and 2500 µg NO_3^- g DW^{-1} day^{-1}, respectively. In comparison to NH_4^+, the specific uptake rate and removal efficiency for NO_3^- were much higher.

Overall, this study has been successful at illustrating the potential of *Ulva sp.* in RAS waste streams by examining the changes in growth, chemical composition and uptake behavior. A potential use of the biomass has been illustrated by the use of an explorative co-culture between *Ulva sp.* and *L. littorea*. The suggestions for future research should be given sufficient attention to improve to overall accuracy of the research and the output of the explorative model.

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7. Appendix A

7.1 Pilot study

Goal and study set up

A pilot study has been conducted in May 2021 to study the vegetative growth of *Ulva* in RAS water in varying light intensities. However, the *Ulva* started to bleach in the first week of the experiment. Therefore, the goal of the study was altered to finding under which light intensity the seaweed biomass showed less bleaching.

The study was set up with 3 treatments and 1 control for the light intensities, each having four replicates. All treatments received RAS waste water. The highest light intensity was approximately 50 µmol m^{-2} s^{-1}. The treatment with medium light intensity had approximately 25 µmol m^{-2} s^{-1} and the low light intensity was approximately 7 µmol m^{-2} s^{-1}. The control received seawater pumped from 70 meters depth and had a medium light intensity of 25 µmol m^{-2} s^{-1} as well. The densities of seaweed was the same in every tank, with 1 kg FW m^{-2}, as this was determined to be the optimal density for the cultivation of *Ulva* (Debusk et al., 1986; Neori et al., 1991).

To analyze the effect of light on *Ulva* in RAS water, sampling occurred on day 13 and day 20 of the experiment. Below, the results for healthy, bleached and total biomass on these two sample moments are discussed.

Table A.1. Overview of the different treatments in pilot study.

	REPLICATE 1	REPLICATE 2	REPLICATE 3	REPLICATE 4	LIGHT INTENSITY
RAS WATER	Tank 1	Tank 2	Tank 3	Tank 4	50 µmol m^{-2} s^{-1}
RAS WATER	Tank 5	Tank 6	Tank 7	Tank 8	25 µmol m^{-2} s^{-1}
RAS WATER	Tank 9	Tank 10	Tank 11	Tank 12	7 µmol m^{-2} s^{-1}
SEA WATER	Tank 13	Tank 14	Tank 15	Tank 16	25 µmol m^{-2} s^{-1}

Results

The healthy biomass (i.e. non bleached) was significantly higher on both sampling day 13 and 20 in the high light intensity tanks. There was no significant difference in the amount of healthy

biomass between the middle, low light intensity and control tanks on day 13. However, on day 20, the tank with middle intensity light had a higher healthy biomass amount than the low intensity light tanks. Furthermore, the control tank, receiving medium light intensity, had a higher healthy biomass amount as well. On both days, the variable of "Light" has a significant effect on both sampling days

On day 13, there was no significant difference in the bleached biomass between all the treatment and control groups. However, on day 20, The high light intensity had a lower bleached biomass than the low light intensity tanks. Furthermore, the high light intensity tank had a lower bleached biomass than the control, receiving seawater and medium light intensity. There was no significant difference between the bleached biomass of the high and medium light intensities and between the medium and low light intensities. The variable "Light" only had a significant effect on sampling day 20.

The total biomass on day 13 and 20 was significantly higher in the high light intensities compared to the low and middle light intensities. The variable of "Light" only had a significant effect on sampling day 20.

Lessons learnt

Overall, the healthy biomass is significantly higher in high light intensity tanks compared to middle, low light intensity and control tanks throughout the experiment. The bleached biomass is significantly higher in low light intensity tanks compared to middle and high light intensity tanks. The total biomass is significantly higher in high light intensity tanks compared to middle and low light intensity tanks. Therefore, the high light intensity tanks performed better than the middle and low light intensity tank. The lesson learnt from this pilot study is that the appropriate light intensity is high, around 60 µmol m^{-2} s^{-1}.

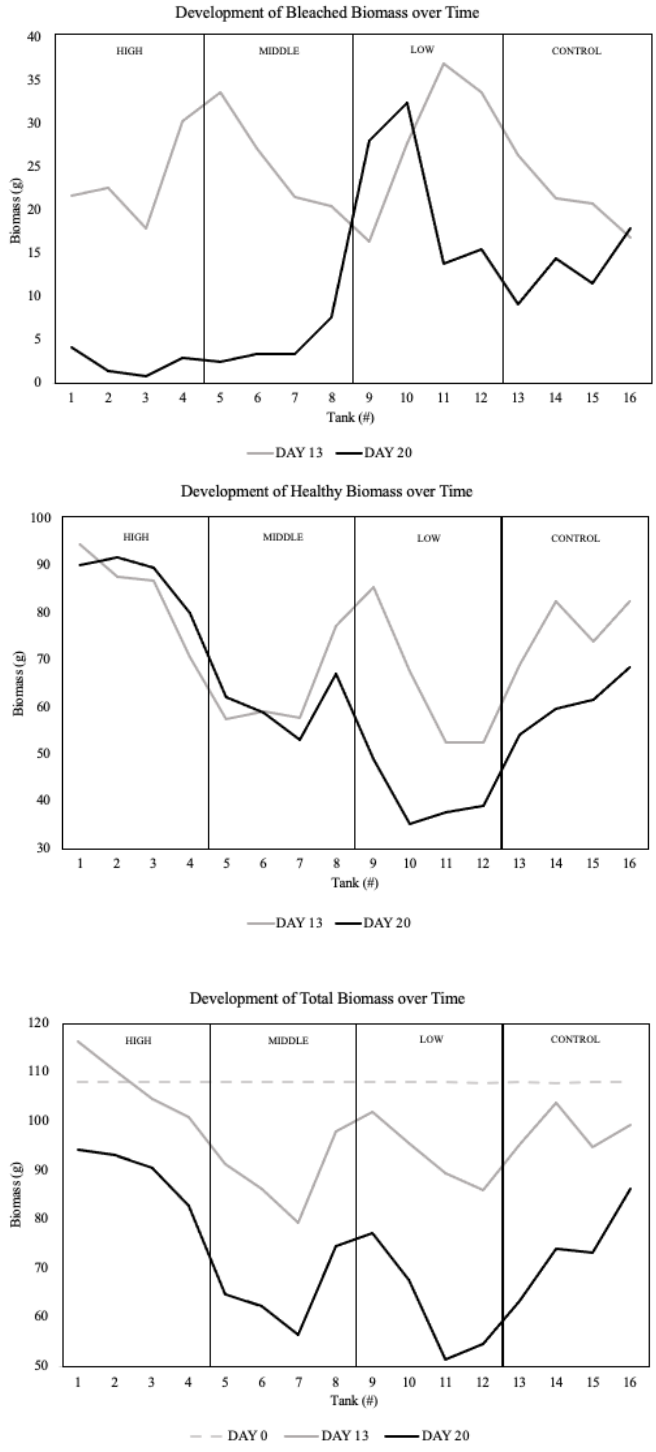

Figure A.1. Development of healthy, bleached and total biomass over time (g)

8. Appendix B: Seaweed Experiment I

Table B.1. Results of Seaweed Experiment I: SGR. The stars in the right column denote the significance codes between: 0 '***' 0,001 '**' 0,01 '*' 0,05. Treatment 1,2,3,4 treatments used in this experiment. Respectively, they denote 100%, 50 %, 25% and 0 % RAS water.

LM(FORMULA = SGR WEIGHT ~ DAY * TREATMENT + PH + LIGHT)					
RESIDUALS:					
	Min	*1Q*	*Median*	*3Q*	*Max*
	-10.05	-1.82	0.25	1.97	10.59
COEFFICIENTS:					
	Estimate	*Std. Error*	*t value*	*Pr(>|t|)*	*Significance level*
INTERCEPT	-48.02	34.69	-1.38	0.171	
DAY	-0.46	0.11	-4.04	<0.001	***
TREATMENT 2	-3.79	2.77	-1.37	0.175	
TREATMENT 3	-4.00	2.74	-1.46	0.149	
CONTROL	-9.55	2.88	-3.31	0.001	**
PH	8.23	3.99	2.06	0.043	*
LIGHT	-0.03	0.17	-0.20	0.846	
DAY : TREATMENT 2	0.21	0.15	1.33	0.187	
DAY: TREATMENT 3	0.30	0.16	1.95	0.055	.
DAY: CONTROL	0.37	0.15	2.38	0.020	*
OTHER					
ADJUSTED R-SQUARED:	0.40				
P-VALUE:	0.00				

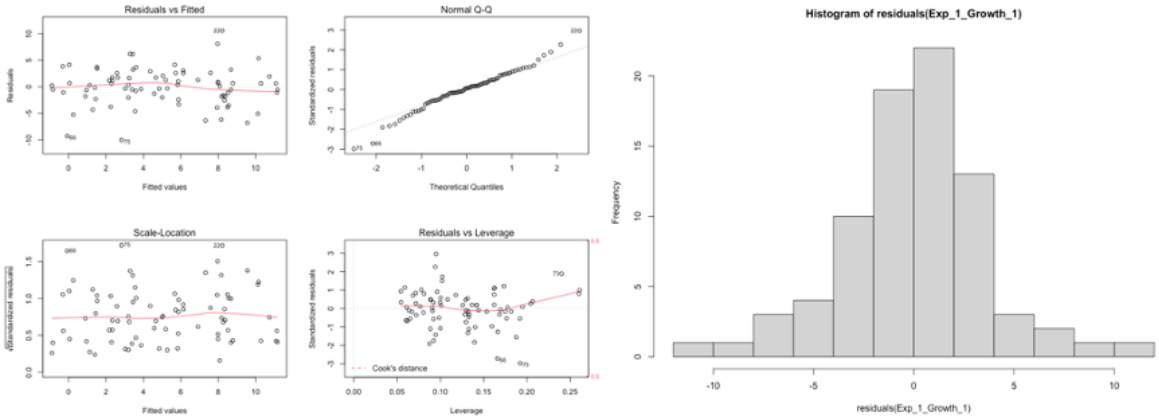

Figure B.1. Fit of the model for SGR of the Seaweed Experiment I with the four diagnostic plots are shown on the left. On the right, a histogram of the residuals of the model is shown.

Table B.2: Results of Seaweed Experiment I: carbon content (µg C mg DW^{-1}) of seaweed tissue. The stars in the right column denote the significance codes between: 0 '***' 0.001 '**' 0.01 '*' 0.05 ',' 0.1 ' ' 1. Treatment 1,2,3,4 treatments used in this experiment. Respectively, they denote 100%, 50 %, 25% and 0 % RAS water.

LM(FORMULA = µG C/ MG DW2 ~ DAY * TREATMENT

RESIDUALS:

	Min	1Q	Median	3Q	Max
	-28.28	-2.47	0.00	0.85	16.55

COEFFICIENTS:

	Estimate	Std. Error	t value	Pr(>|t|)	Significance level
(INTERCEPT)	332.20	4.56	72.87	< 2e-16	***
DAY 6	20.74	6.45	3.22	0.003	**
DAY 27	4.59	6.45	0.71	0.482	
TREATMENT 3	0.00	6.45	0.00	1.000	
TREATMENT 2	0.00	6.45	0.00	1.000	
TREATMENT 4	0.00	6.45	0.00	1.000	
DAY 6: TREATMENT 3	0.11	9.12	0.01	0.991	
DAY 27: TREATMENT 3	11.16	9.12	1.22	0.230	
DAY 6: TREATMENT 2	-8.27	9.49	-0.87	0.390	
DAY 27: TREATMENT 2	30.17	9.12	3.31	0.002	**
DAY 6: TREATMENT 4	9.03	12.06	0.75	0.459	
DAY 27: TREATMENT 4	16.94	9.12	1.86	0.072	

OTHER

ADJUSTED R-SQUARED:	0.5846
P-VALUE:	1.50E-05

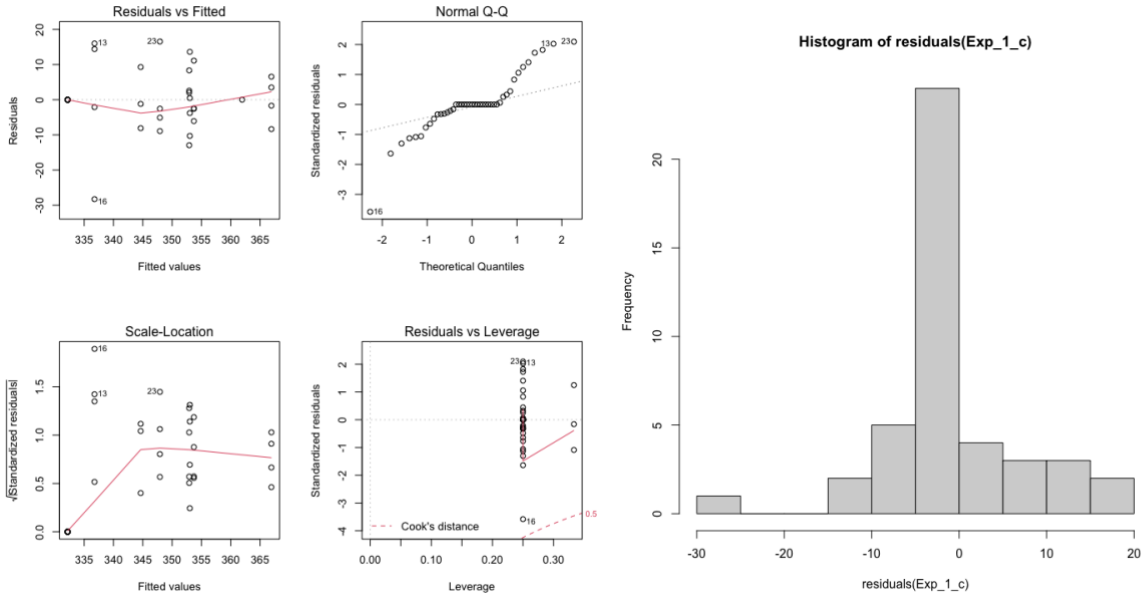

Figure B.2. Fit of the model for carbon content of the Seaweed Experiment I with the four diagnostic plots are shown on the left. On the right, a histogram of the residuals of the model is shown.

Table B.3: Results of Seaweed Experiment I: nitrogen content (µg N mg DW^{-1}) of seaweed tissue. The stars in the right column denote the significance codes between: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. Treatment 1,2,3,4 treatments used in this experiment. Respectively, they denote 100%, 50 %, 25% and 0 % RAS water.

LM(FORMULA = µG N/ MG DW2 ~ DAY * TREATMENT)					
RESIDUALS:					
	Min	1Q	Median	3Q	Max
	-5,97	-0,48	0,00	0,23	6,39
COEFFICIENTS:					
	Estimate	Std. Error	t value	Pr(>|t|)	Significance level
(INTERCEPT)	26,25	1,36	19,24	< 2e-16	***
DAY 6	15,05	1,93	7,80	0,000	***
DAY 27	23,88	1,93	12,38	0,000	***
TREATMENT 2	0,00	1,93	0,00	1,000	
TREATMENT 3	0,00	1,93	0,00	1,000	
TREATMENT 4	0,00	1,93	0,00	1,000	
DAY 6: TREATMENT 2	-3,82	2,73	-1,40	0,171	
DAY 27: TREATMENT 2	2,59	2,73	0,95	0,349	
DAY 6: TREATMENT 3	0,38	2,84	0,13	0,895	
DAY 27: TREATMENT 3	1,11	2,73	0,41	0,687	
DAY 6: TREATMENT 4	-19,85	3,61	-5,50	0,000	***
DAY 27: TREATMENT 4	-33,77	2,73	-12,38	0,000	***
OTHER:					
ADJUSTED R-SQUARED:	0,9513				
P-VALUE:	< 2,2e-16				

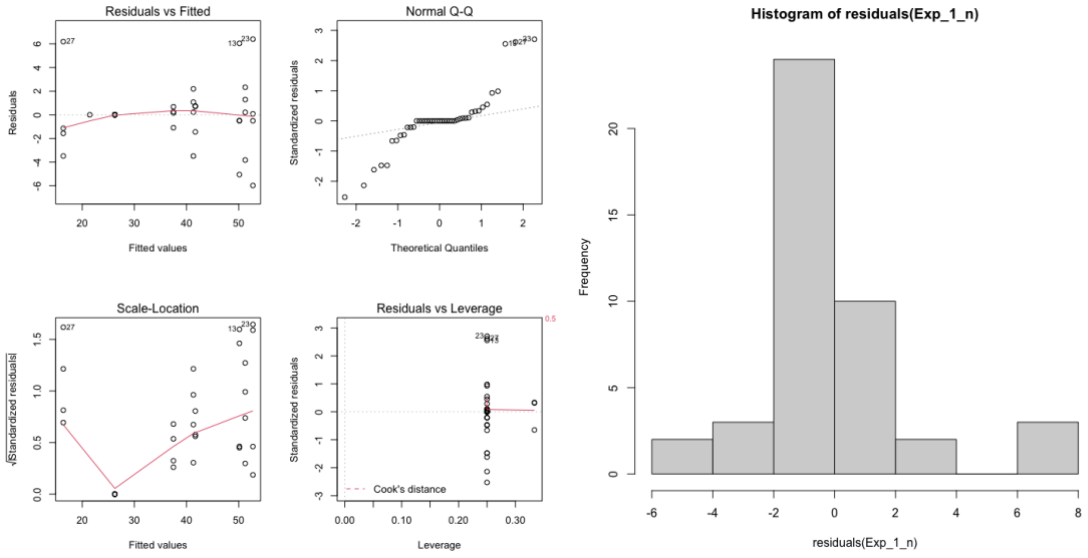

Figure B.3. Fit of the model for nitrogen content of the Seaweed Experiment I with the four diagnostic plots are shown on the left. On the right, a histogram of the residuals of the model is shown.

Table B.4: Results of Seaweed Experiment I: C:N of seaweed tissue. The stars in the right column denote the significance codes between: 0 '***' 0,001 '**' 0,01 '*' 0,05 ',' 0,1 ' ' 1. Treatment 1,2,3,4 treatments used in this experiment. Respectively, they denote 100%, 50 %, 25% and 0 % RAS water.

LM(FORMULA = C:N ~ DAY * TREATMENT)					
RESIDUALS:					
	Min	*1Q*	*Median*	*3Q*	*Max*
	-6.3181	-0.1043	0	0.0839	4.7578
COEFFICIENTS:					
	Estimate	Std. Error	t value	Pr(>|t|)	Significance level
(INTERCEPT)	12.65	0.86	14.63	<0.001	***
DAY 6	-4.08	1.22	-3.34	0.002	**
DAY 27	-5.92	1.22	-4.84	<0.001	***
TREATMENT2	0.00	1.22	0.00	1.000	
TREATMENT3	0.00	1.22	0.00	1.000	
CONTROL	0.00	1.22	0.00	1.000	
DAY 6: TREATMENT 2	-0.30	1.80	-0.17	0.870	
DAY 27: TREATMENT 2	0.44	1.73	0.25	0.802	
DAY 6 : TREATMENT3	0.91	1.80	0.51	0.616	
DAY 27 : TREATMENT 3	-0.10	1.73	-0.06	0.953	
DAY 6 : CONTROL	4.50	1.93	2.33	0.027	*
DAY 27 : CONTROL	15.76	1.73	9.11	<0.001	***
OTHER					
ADJUSTED R-SQUARED:	0.863				
P-VALUE:	0.000				

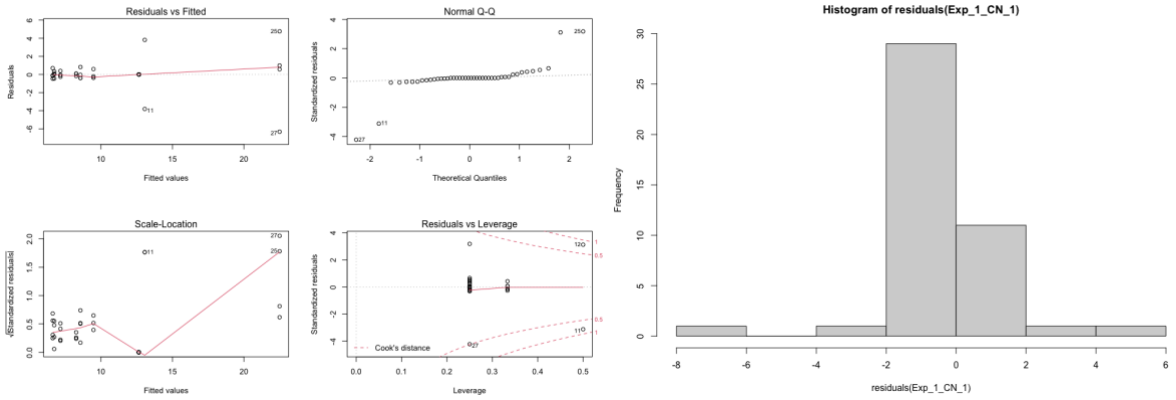

Figure B.4. Fit of the model for C:N ratio of the Seaweed Experiment I with the four diagnostic plots are shown on the left. On the right, a histogram of the residuals of the model is shown.

The fit of the model for the protein content has been improved by deleting data point 11 and 12 that were part of treatment 3 and 4, respectively. These data points were given as outliers with too much leverage on the model outputs by the diagnostic plots. This has improved the R^2 with 0.04 and has lowered the AIC with 30 points.

Table B.5. Results of Seaweed Experiment I: protein content of seaweed tissue. The stars in the right column denote the significance codes between: 0 '***' 0,001 '**' 0,01 '*' 0,05 ',' 0,1 ' ' 1. Treatment 1,2,3,4 treatments used in this experiment. Respectively, they denote 100%, 50 %, 25 and 0 % RAS water.

LM(FORMULA = PROTEIN CONTENT ~ DAY * TREATMENT)					
RESIDUALS:					
	Min	*1Q*	*Median*	*3Q*	*Max*
	-3.55	-0.22	0.00	0.16	3.55
COEFFICIENTS:					
	Estimate	Std. Error	t value	Pr(>|t|)	Significance level
(INTERCEPT)	11.46	0.74	15.43	<0.001	***
DAY 6	6.57	1.05	6.25	<0.001	***
DAY 27	10.42	1.05	9.92	<0.001	***
TREATMENT2	0.00	1.05	0.00	1.00	
TREATMENT3	0.00	1.05	0.00	1.00	
CONTROL	0.00	1.05	0.00	1.00	
DAY 6: TREATMENT 2	0.16	1.55	0.11	0.916	
DAY 27: TREATMENT 2	0.48	1.49	0.33	0.747	
DAY 6 : TREATMENT3	-1.70	1.55	-1.10	0.279	
DAY 27 : TREATMENT 3	1.13	1.49	0.76	0.452	
DAY 6 : CONTROL	-5.11	1.66	-3.08	0.04	**
DAY 27 : CONTROL	-14.74	1.49	-9.92	<0.001	***
OTHER					
ADJUSTED R-SQUARED:		0.92			
P-VALUE:		0.00			

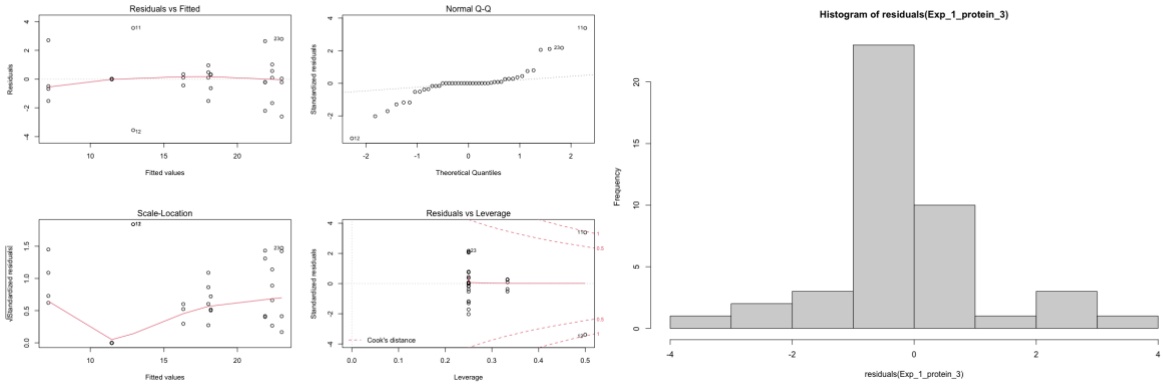

Figure B.5. Fit of the model for protein content of the Seaweed Experiment I

Table B.6: Results of Seaweed Experiment I: Organic phosphates (OP) level of seaweed tissue. The stars in the right column denote the significance codes between: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. The RAS 1,2,3,4 treatments used in this experiment. Respectively, they denote 100%, 50 %, 25% and 0 % RAS water.

LM(FORMULA = OP ~ DAY * TREATMENT)					
RESIDUALS:					
	Min	*1Q*	*Median*	*3Q*	*Max*
	0.60	-0.05	0.00	0.01	1.11
COEFFICIENTS:					
	Estimate	Std. Error	t value	Pr(>|t|)	Significance level
(INTERCEPT)	0.57	4.05	0.14	0.89	
DAY 6	1.24	0.27	4.68	<0.001	***
DAY 27	-0.01	0.28	5.78	<0.001	***
TREATMENT2	0.00	0.28	-0.04	0.965	
TREATMENT3	0.01	0.30	0.02	0.989	
CONTROL	0.01	0.02	0.04	0.968	
DAY 6: TREATMENT 2	-0.21	0.38	0.79	0.431	
DAY 27: TREATMENT 2	-0.50	0.38	-1.32	0.200	
DAY 6 : TREATMENT3	-0.82	0.38	-2.18	0.04	*
DAY 27 : TREATMENT 3	-0.48	0.38	-1.75	0.212	
DAY 6 : CONTROL	-1.46	0.50	-2.94	0.001	**
DAY 27 : CONTROL	-2.02	0.38	-5.37	<0.001	***
OTHER					
ADJUSTED R-SQUARED:	0.73				
P-VALUE:	0.000				

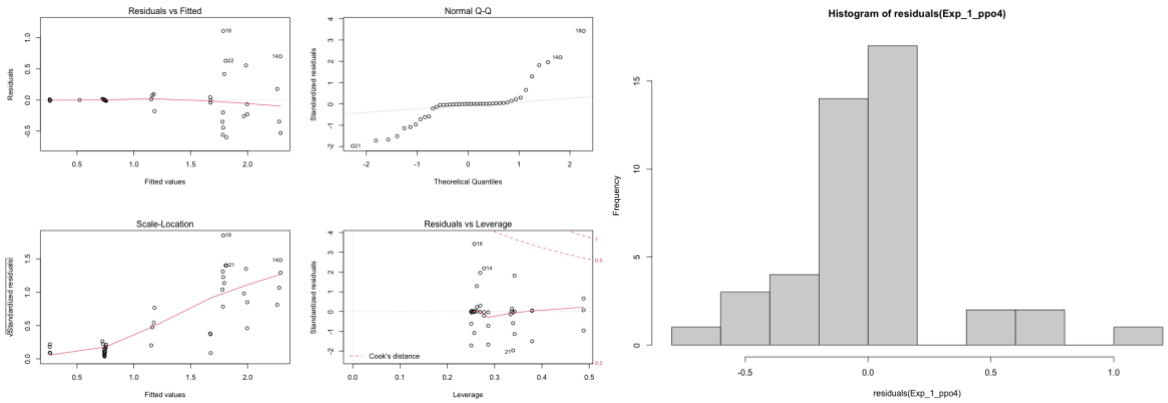

Figure B.6. Fit of the model for OP of the Seaweed Experiment I

Table B.7: Results of Seaweed Experiment I: N:P ratio level of seaweed tissue. The stars in the right column denote the significance codes between: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. The RAS 1,2,3,4 treatments used in this experiment. Respectively, they denote 100%, 50 %, 25% and 0 % RAS water.

LM(FORMULA = N:P ~ DAY * TREATMENT)					
RESIDUALS:					
	Min	*1Q*	*Median*	*3Q*	*Max*
	-13.35	-2.01	0.00	0.21	21.97
COEFFICIENTS:					
	Estimate	Std. Error	t value	Pr(>|t|)	Significance level
(INTERCEPT)	35.39	3.31	10.69	<0.001	***
DAY 6	-14.18	4.68	-3.03	0.005	**
DAY 27	-12.28	4.68	-2.62	0.013	*
TREATMENT2	0.00	4.68	0.00	1.000	
TREATMENT3	0.00	4.68	0.00	1.000	
CONTROL	0.00	4.68	0.00	1.000	
DAY 6: TREATMENT 2	3.71	6.89	0.54	0.594	
DAY 27: TREATMENT 2	8.75	6.62	1.32	0.196	
DAY 6 : TREATMENT3	11.18	6.62	1.69	0.101	
DAY 27 : TREATMENT 3	8.39	6.62	1.27	0.214	
DAY 6 : CONTROL	19.77	8.76	2.26	0.031	*
DAY 27 : CONTROL	39.90	6.62	5.94	<0.001	***
OTHER					
ADJUSTED R-SQUARED:	0.70				
P-VALUE:	0.000				

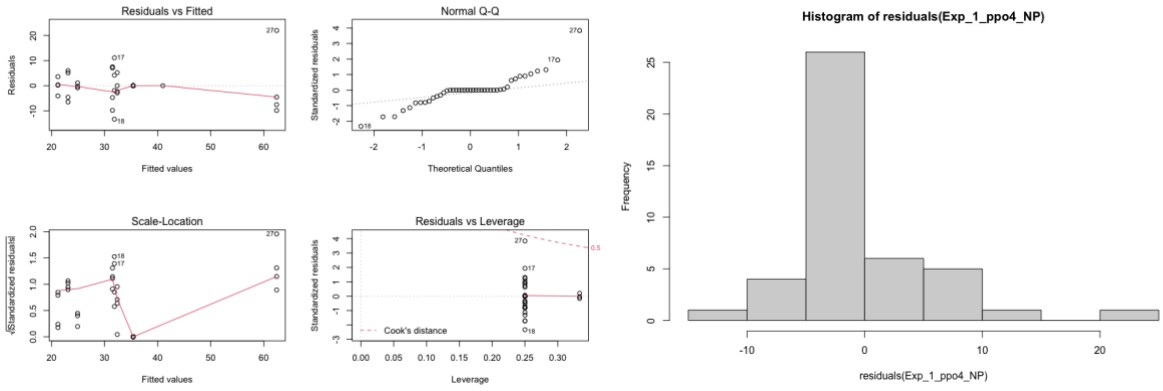

Figure B.7. Fit of the model for N:P of the Seaweed Experiment I

Table B.8: Results of Seaweed Experiment I: NH_4^+water stability. The stars in the right column denote the significance codes between: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. The RAS 1,2,3,4 treatments used in this experiment. Respectively, they denote 100%, 50 %, 25% and 0 % RAS water.

LM(FORMULA = NH_4^+ ~ DAY + TREATMENT)					
RESIDUALS:					
	Min	*1Q*	*Median*	*3Q*	*Max*
	-70.34	-15.65	4.15	11.63	113.81
COEFFICIENTS:					
	Estimate	Std. Error	t value	Pr(>|t|)	Significance level
(INTERCEPT)	208.64	11.38	18.33	<0.001	***
DAY 1	-61.47	11.38	-5.40	<0.001	***
DAY 2	-68.82	11.38	-6.04	<0.001	
TREATMENT2	-3.87	13.14	-0.30	0.77	
TREATMENT3	-11.35	13.14	-0.86	0.393	
CONTROL	-16.63	13.14	-1.27	0.213	
OTHER					
ADJUSTED R-SQUARED:	0.47				
P-VALUE:	0.000				

Table B.9: Results of Seaweed Experiment I: NO_2^- water stability. The stars in the right column denote the significance codes between: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. The RAS 1,2,3,4 treatments used in this experiment. Respectively, they denote 100%, 50 %, 25% and 0 % RAS water.

LM(FORMULA = NO_2^- ~ DAY + TREATMENT)					
RESIDUALS:					
	Min	*1Q*	*Median*	*3Q*	*Max*
	-13.42	-5.47	-1.69	3.59	33.31
COEFFICIENTS:					
	Estimate	Std. Error	t value	Pr(>|t|)	Significance level
(INTERCEPT)	17.00	3.02	5.62	<0.001	***
DAY 1	8.60	3.02	2.84	0.007	**
DAY 2	13.46	3.02	4.45	<0.001	***
TREATMENT2	-5.84	3.49	-1.67	0.102	
TREATMENT3	-10.75	3.49	-3.08	0.004	**
CONTROL	19.24	3.49	-5.11	<0.001	***
OTHER					
ADJUSTED R-SQUARED:	0.51				
P-VALUE:	<0.001				

Table B.10: Results of Seaweed Experiment I: NO3 water stability. The stars in the right column denote the significance codes between: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. The RAS 1,2,3,4 treatments used in this experiment. Respectively, they denote 100%, 50 %, 25% and 0 % RAS water.

LM(FORMULA = NO_3^- ~ DAY + TREATMENT)					
RESIDUALS:					
	Min	*1Q*	*Median*	*3Q*	*Max*
	-15269.6	-3275.4	-883.2	5397.6	8077.1
COEFFICIENTS:					
	Estimate	Std. Error	t value	Pr(>|t|)	Significance level
(INTERCEPT)	31300	2422	12.92	<0.001	***
DAY 1	-23390	2422	-9.66	<0.001	***
DAY 2	-22419	2422	-9.26	<0.001	***
TREATMENT2	-1168	2797	-0.42	0.678	
TREATMENT3	-2149	2797	-0.77	0.447	
CONTROL	-15008	2797	-5.37	<0.001	***
OTHER					
ADJUSTED R-SQUARED:	0.76				
P-VALUE:	<0.001				

Table B.11: Results of Seaweed Experiment I: PO_4^{3-}water stability. The stars in the right column denote the significance codes between: 0 '***' 0,001 '**' 0,01 '*' 0,05 ',' 0,1 ' ' 1. The RAS 1,2,3,4 treatments used in this experiment. Respectively, they denote 100%, 50 %, 25% and 0 % RAS water.

LM(FORMULA = PO_4^{3-} ~ DAY + TREATMENT)					
RESIDUALS:					
	Min	*1Q*	*Median*	*3Q*	*Max*
	-543.11	-168.06	-67.16	301.22	389.26
COEFFICIENTS:					
	Estimate	Std. Error	t value	Pr(>|t|)	Significance level
(INTERCEPT)	1551.10	107.10	14.48	<0.001	***
DAY 1	-857.9	107.10	-8.01	<0.001	***
DAY 2	-771.40	107.10	-7.20	<0.001	***
TREATMENT2	440.30	123.70	-3.56	<0.001	***
TREATMENT3	-520.10	123.70	-4.21	<0.001	***
CONTROL	-1003.80	123.70	-8.11	<0.001	***
OTHER					
ADJUSTED R-SQUARED:	0.75				
P-VALUE:	<0.001				

9. Appendix C: Seaweed Experiment II

The growth rate of the seaweed is included to ensure that the specific uptake per g included the growth of the seaweed over time. This data set contains n=16 samples. The data set contains only the variable of treatment to explain the growth rate in weight of seaweed. Overall, there are no significant differences between the treatments and the artificial seawater. The average growth rates are summarized in Table 12. These average growth rates are applied in the uptake rates of the nutrients described below.

Table C.1: Results of Seaweed Experiment II: Growth rate. The stars in the right column denote the significance codes between: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. The density treatments 5-8 used in this experiment. Respectively, they denote 100% (RAS), 200 % (RAS), 300% (RAS) and 100 % (Artificial seawater) biomass.

LM(FORMULA = GROWTH RATE ~ DAY * TREATMENT)					
RESIDUALS:					
	Min	*1Q*	*Median*	*3Q*	*Max*
	-0.02	-0.01	0.00	0.00	0.003
COEFFICIENTS:					
	Estimate	Std. Error	t value	Pr(>|t|)	Significance level
(INTERCEPT)	1.01	0.00	165.55	<0.001	***
TREATMENT 6	0.01	0.00	1.58	0.141	
TREATMENT 7	0.02	0.00	0.06	0.063	
CONTROL	0.00	0.00	0.71	0.713	
OTHER					
ADJUSTED R-SQUARED:	0.26				
P-VALUE:	0.09				

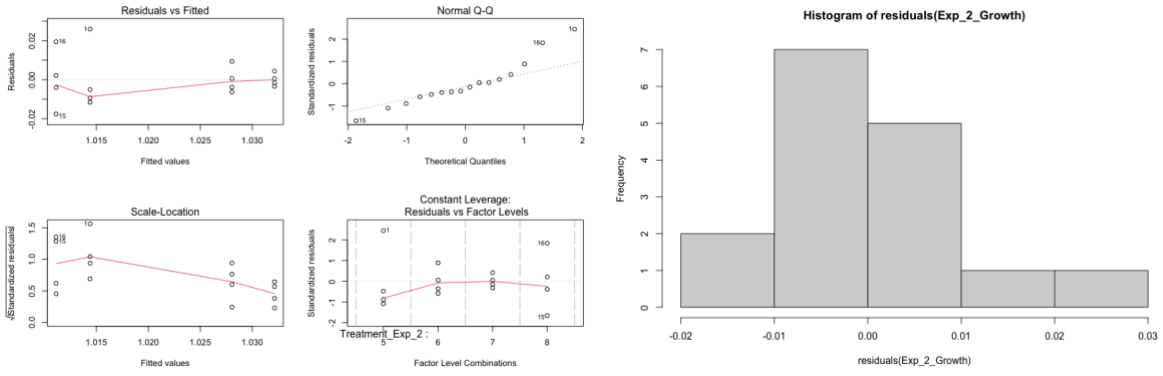

Figure C.1. Fit of the model for growth rate of the Seaweed Experiment I I

Table C.2: Results of Seaweed Experiment II: Specific Uptake rate NH_4^+. The stars in the right column denote the significance codes between: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. The density treatments 5-8 used in this experiment. Respectively, they denote 100% (RAS), 200 % (RAS), 300% (RAS) and 100 % (Artificial seawater) biomass.

LM(FORMULA = V NH_4^+ ~ DAY * TREATMENT)					
RESIDUALS:					
	Min	*1Q*	*Median*	*3Q*	*Max*
	-8.41	-1.77	0.69	2.17	7.80
COEFFICIENTS:					
	Estimate	Std. Error	t value	Pr(>|t|)	Significance level
(INTERCEPT)	20.50	2.10	9.76	<0.001	***
TREATMENT 6	-11.13	2.97	-3.47	0.002	**
TREATMENT 7	-13.59	2.97	-4.57	0.001	***
CONTROL	-15.66	2.97	-5.27	<0.001	***
OTHER					
ADJUSTED R-SQUARED:	0.67				
P-VALUE:	0.001				

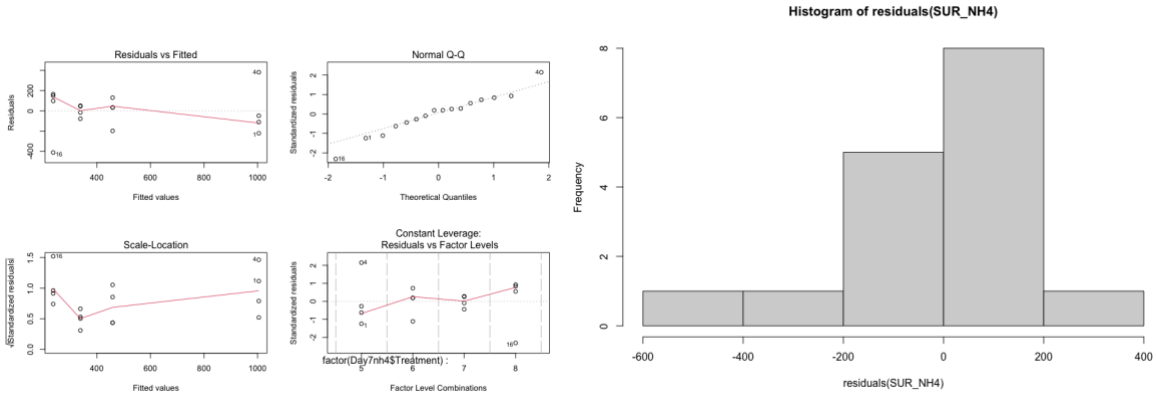

Figure C.2. Fit of the model for V- NH_4^+ of the Seaweed Experiment II

Table C.3: Results of Seaweed Experiment II: Specific Uptake rate N02. The stars in the right column denote the significance codes between: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. The density treatments 5-8 used in this experiment. Respectively, they denote 100% (RAS), 200 % (RAS), 300% (RAS) and 100 % (Artificial seawater) biomass.

LM(FORMULA = VNO_2^- ~ DAY * TREATMENT)					
RESIDUALS:					
	Min	*1Q*	*Median*	*3Q*	*Max*
	-2.53	-0.24	0.04	0.29	2.67
COEFFICIENTS:					
	Estimate	Std. Error	t value	Pr(>|t|)	Significance level
(INTERCEPT)	-1.85	0.66	-2.82	0.016	*
TREATMENT 6	1.45	0.93	1.57	0.143	
TREATMENT 7	2.10	0.93	2.26	0.043	*
CONTROL	1.90	0.93	2.01	0.062	
OTHER					
ADJUSTED R-SQUARED:	0.18				
P-VALUE:	0.015				

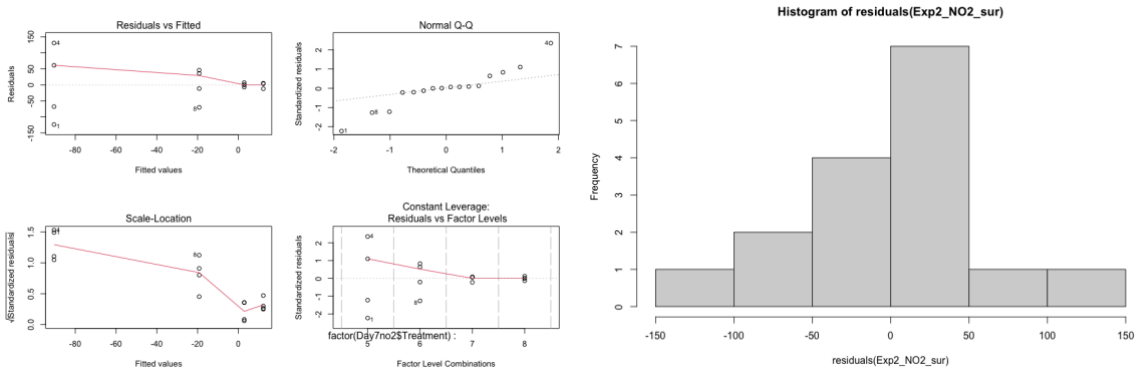

Figure C.3. Fit of the model for V-NO_2^- of the Seaweed Experiment II

Table C.4: Results of Seaweed Experiment II: Specific Uptake rate NO_3^-. The stars in the right column denote the significance codes between: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. The density treatments 5-8 used in this experiment. Respectively, they denote 100% (RAS), 200 % (RAS), 300% (RAS) and 100 % (Artificial seawater) biomass.

LM(FORMULA = VNO_3^- ~ DAY * TREATMENT)					
RESIDUALS:					
	Min	*1Q*	*Median*	*3Q*	*Max*
	-648.30	-122.68	-41.69	109.92	1242.45
COEFFICIENTS:					
	Estimate	Std. Error	t value	Pr(>|t|)	Significance level
(INTERCEPT)	5556.1	229.2	24.24	<0.001	***
TREATMENT 6	-1707.1	324.2	-5.27	<0.001	***
TREATMENT 7	-3029.1	324.2	-9.34	<0.001	***
CONTROL	-7768.2	324.2	-23.96	<0.001	***
OTHER					
ADJUSTED R-SQUARED:	0.98				
P-VALUE:	<0.001				

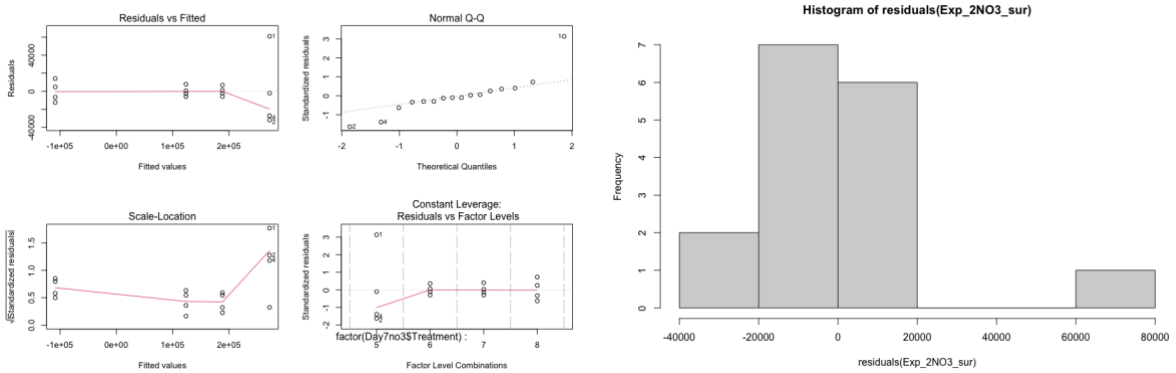

Figure C.4. Fit of the model for V- NO_3^- of the Seaweed Experiment II

The data for the specific uptake rate of PO_4^{3-} shows to not the normally distributed by looking at the distribution of the residuals and the diagnostic plots. The data points, 18, 33 and 36 show to be outliers. All these data points occur in treatment 5. When deleting the datapoints from dataset, the normal distribution of the residuals improves, as well as the adjusted R^2. Therefore, it is decided to use the altered dataset.

Table C.5: Results of Seaweed Experiment II: Specific Uptake rate PO_4^{3-}. The stars in the right column denote the significance codes between: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. The density treatments 5-8 used in this experiment. Respectively, they denote 100% (RAS), 200 % (RAS), 300% (RAS) and 100 % (Artificial seawater) biomass.

LM(FORMULA = V PO_4^{3-} ~ DAY * TREATMENT)					
RESIDUALS:					
	Min	*1Q*	*Median*	*3Q*	*Max*
	-44.86	-2.17	-0.51	2.75	44.86
COEFFICIENTS:					
	Estimate	Std. Error	t value	Pr(>|t|)	Significance level
(INTERCEPT)	146.72	14.46	10.15	<0.001	***
TREATMENT 6	-126.31	17.71	-7.13	<0.001	***
TREATMENT 7	-134.91	17.71	-7.62	<0.001	***
CONTROL	-143.23	17.71	-8.09	<0.001	***
OTHER					
ADJUSTED R-SQUARED:	0.85				
P-VALUE:	<0.001				

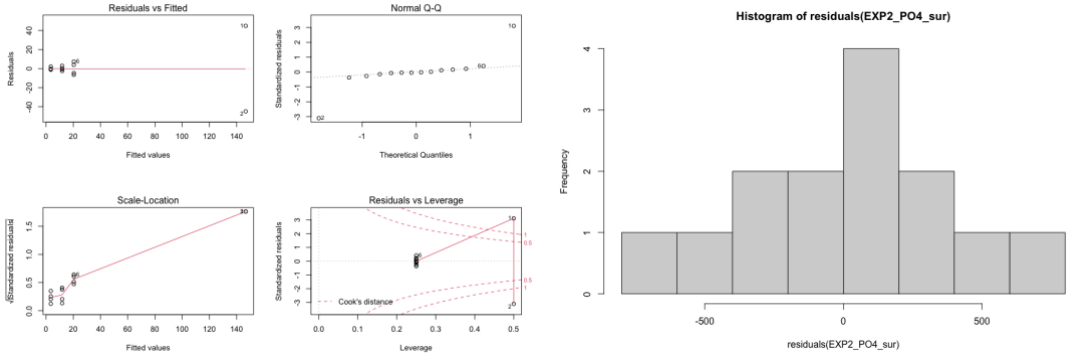

Figure C.5. Fit of the model for V- PO_4^{3-} of the Seaweed Experiment II

Table C.6: Results of Seaweed Experiment II: Removal efficiency rate NH_4^+. The stars in the right column denote the significance codes between: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. The density treatments 5-8 used in this experiment. Respectively. they denote 100% (RAS), 200 % (RAS), 300% (RAS) and 100 % (Artificial seawater) biomass.

LM(FORMULA = RE NH_4^+ ~ TREATMENT)					
RESIDUALS:					
	Min	1Q	Median	3Q	Max
	-17.56	-4.978	1.965	4.89	10.54
COEFFICIENTS:					
	Estimate	Std. Error	t value	Pr(>|t|)	Significance level
(INTERCEPT)	33.07	4.59	7.205	1.08E-05	***
TREATMENT 6	-0.688	6.491	-0.106	0.91735	
TREATMENT 7	2.385	6.491	0.367	0.71976	
TREATMETN 8	-23.352	6.491	-3.597	0.00366	**
OTHER					
ADJUSTED R-SQUARED:	0.54				
P-VALUE:	0.006022				

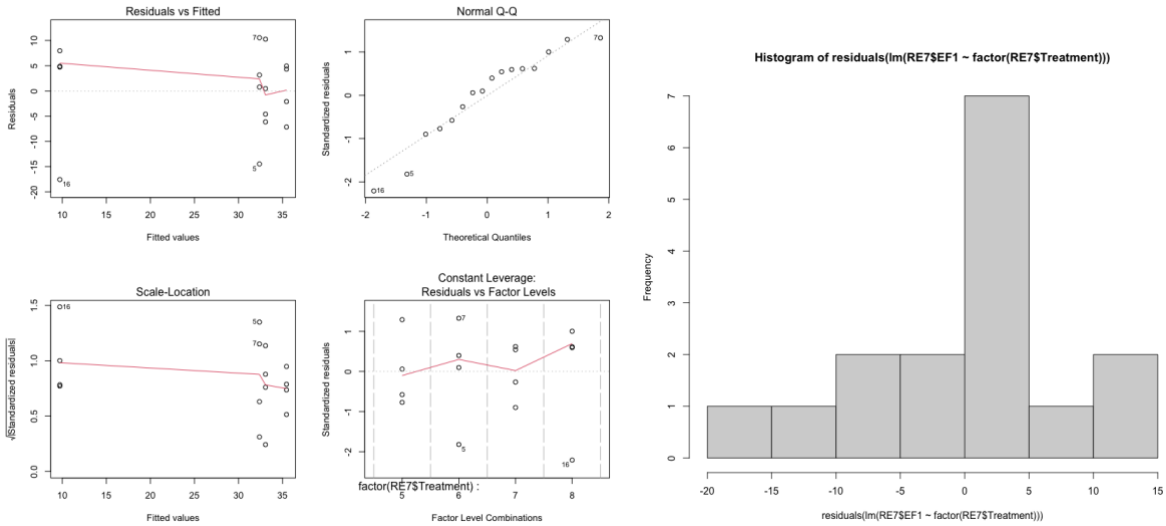

Figure C.6. Fit of the model for Removal efficiency $NH_4^+$of the Seaweed Experiment II

Table C.7. Results of Seaweed Experiment II: Removal efficiency rate No_2^-. The stars in the right column denote the significance codes between: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. The density treatments 5-8 used in this experiment. Respectively, they denote 100% (RAS), 200 % (RAS), 300% (RAS) and 100 % (Artificial seawater) biomass.

LM(FORMULA = $RENO_2^-$ ~ TREATMENT)					
RESIDUALS:					
	Min	1Q	Median	3Q	Max
	-114.42	-23.19	4.36	21.89	105.75
COEFFICIENTS:					
	Estimate	Std. Error	t value	Pr(>|t|)	Significance level
(INTERCEPT)	-75.62	32.87	-2.30	0.040	*
TREATMENT 6	45.72	46.48	0.98	0.345	
TREATMENT 7	106.56	46.48	0.29	0.041	*
TREATMETN 8	79.24	46.48	1.71	0.114	
OTHER					
ADJUSTED R-SQUARED:	0.16				
P-VALUE:	0.175				

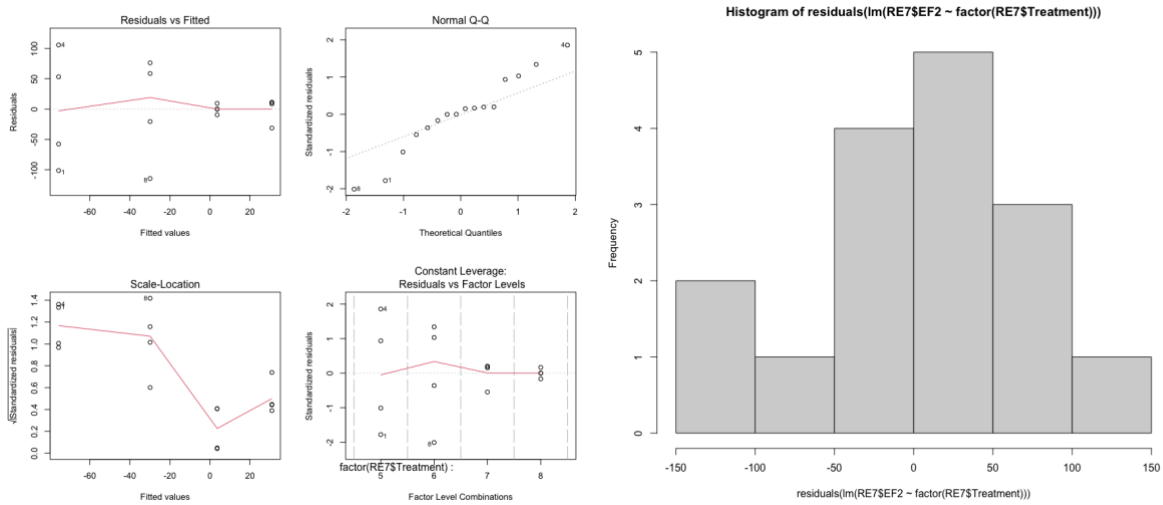

Figure C.7. Fit of the model for Removal efficiency $No_2^-$of the Seaweed Experiment II

Table C.8. Results of Seaweed Experiment II: Removal efficiency rate No_3^-. The stars in the right column denote the significance codes between: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. The density treatments 5-8 used in this experiment. Respectively, they denote 100% (RAS), 200 % (RAS), 300% (RAS) and 100 % (Artificial seawater) biomass.

LM(FORMULA = $RENO_3^-$ ~ TREATMENT)					
RESIDUALS:					
	Min	1Q	Median	3Q	Max
	-56.82	-3.13	1.17	2.60	51.92
COEFFICIENTS:					
	Estimate	Std. Error	t value	Pr(>|t|)	Significance level
(INTERCEPT)	55.62	11.50	4.84	<0.001	***
TREATMENT 6	25.52	16.26	1.57	0.142	
TREATMENT 7	24.02	16.26	1.48	0.165	
TREATMETN 8	-742.48	16.26	-45.69	<0.001	***
OTHER					
ADJUSTED R-SQUARED:	0,99				
P-VALUE:	<0.001				

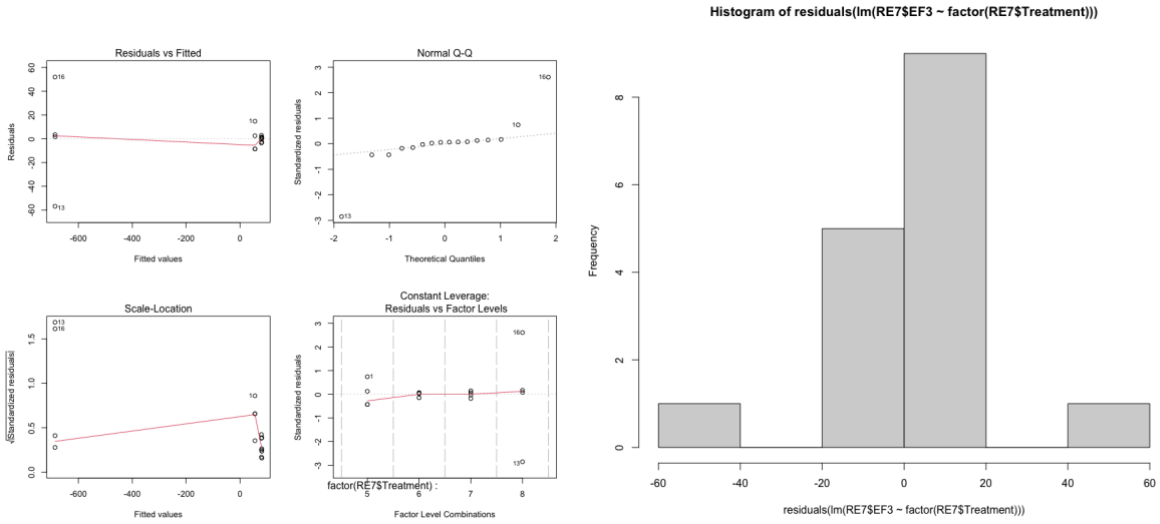

Figure C.8. Fit of the model for Removal efficiency $No_3^-$of the Seaweed Experiment II

Table C.9. Results of Seaweed Experiment II: Removal efficiency rate PO_4^{3-}. The stars in the right column denote the significance codes between: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. The density treatments 5-8 used in this experiment. Respectively, they denote 100% (RAS), 200 % (RAS), 300% (RAS) and 100 % (Artificial seawater) biomass.

LM(FORMULA = RE PO_4^{3-} ~ TREATMENT)					
RESIDUALS:					
	Min	1Q	Median	3Q	Max
	-53.47	-0.48	0.01	1.16	28.49
COEFFICIENTS:					
	Estimate	Std. Error	t value	Pr(>|t|)	Significance level
(INTERCEPT)	91.26	9.21	9.91	<0.001	***
TREATMENT 6	7.72	13.02	0.59	0,564	
TREATMENT 7	8.15	13.02	0.63	0.543	
TREATMETN 8	-77.41	13.02	-5.94	<0.001	***
OTHER					
ADJUSTED R-SQUARED:	0,80				
P-VALUE:	<0.001				

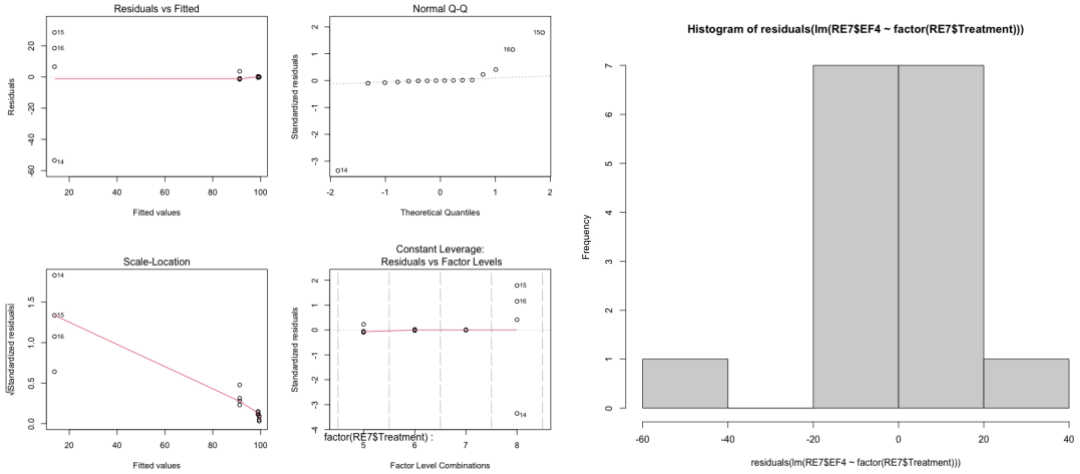

Figure C.9. Fit of the model for Removal efficiency PO_4^{3-} of the Seaweed Experiment II

Table C.10. Results of Seaweed Experiment II: Nutrients in beaker NH_4^+Day 7. The stars in the right column denote the significance codes between: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. The density treatments 5-8 used in this experiment. Respectively. they denote 100% (RAS), 200 % (RAS), 300% (RAS) and 100 % (Artificial seawater) biomass.

LM(FORMULA = NH_4^+~ TREATMENT)					
RESIDUALS:					
	Min	1Q	Median	3Q	Max
	-22.81	-9.67	4.25	10.77	31.31
COEFFICIENTS:					
	Estimate	Std. Error	t value	Pr(>|t|)	Significance level
(INTERCEPT)	144.83	8.86	16.35	<0.001	***
TREATMENT 6	1.48	12.52	0.12	0.907	
TREATMENT 7	-5.16	12.52	-0.41	0.688	
TREATMETN 8	-6.30	12.52	0.05	0.627	
OTHER					
ADJUSTED R-SQUARED:	-0.195				
P-VALUE:	0.091				

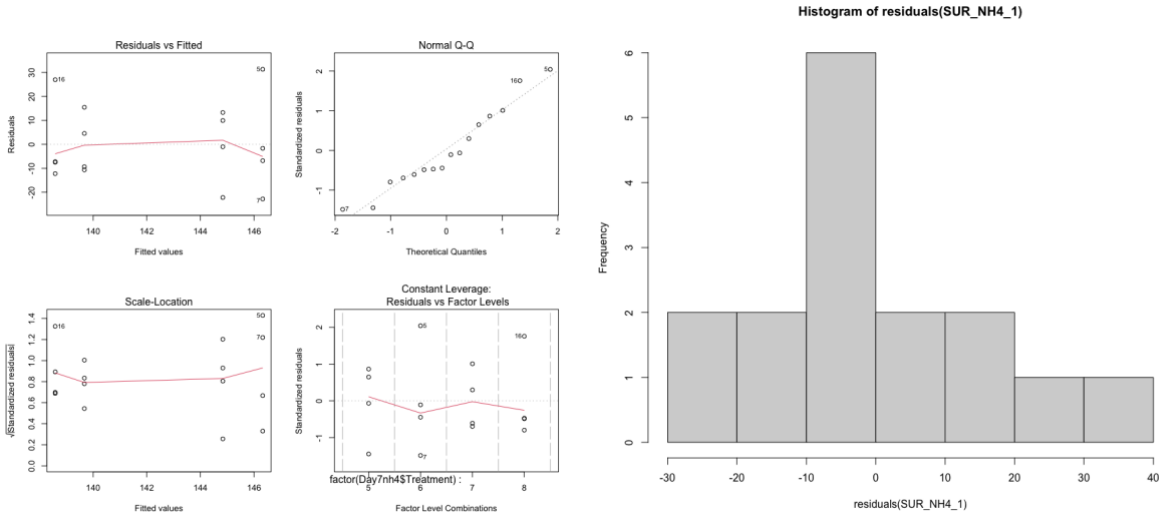

Figure C.10. Fit of the model for Nutrient left in beaker $NH_4^+$of the Seaweed Experiment II

Table C.11. Results of Seaweed Experiment II: Nutrients in beaker NO_2^- Day 7. The stars in the right column denote the significance codes between: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. The density treatments 5-8 used in this experiment. Respectively, they denote 100% (RAS), 200 % (RAS), 300% (RAS) and 100 % (Artificial seawater) biomass.

LM(FORMULA = NO_2^-~ TREATMENT)					
RESIDUALS:					
	Min	1Q	Median	3Q	Max
	-9.54	-1.97	-0.24	2.09	10.32
COEFFICIENTS:					
	Estimate	Std. Error	t value	Pr(>|t|)	Significance level
(INTERCEPT)	15.84	2.96	5.35	<0.001	***
TREATMENT 6	-4.13	4.19	-0.99	0.344	
TREATMENT 7	-9.61	4.19	-2.30	0.04	*
TREATMETN 8	-10.92	4.19	-2.61	0.023	*
OTHER					
ADJUSTED R-SQUARED:	0.28				
P-VALUE:	0.077				

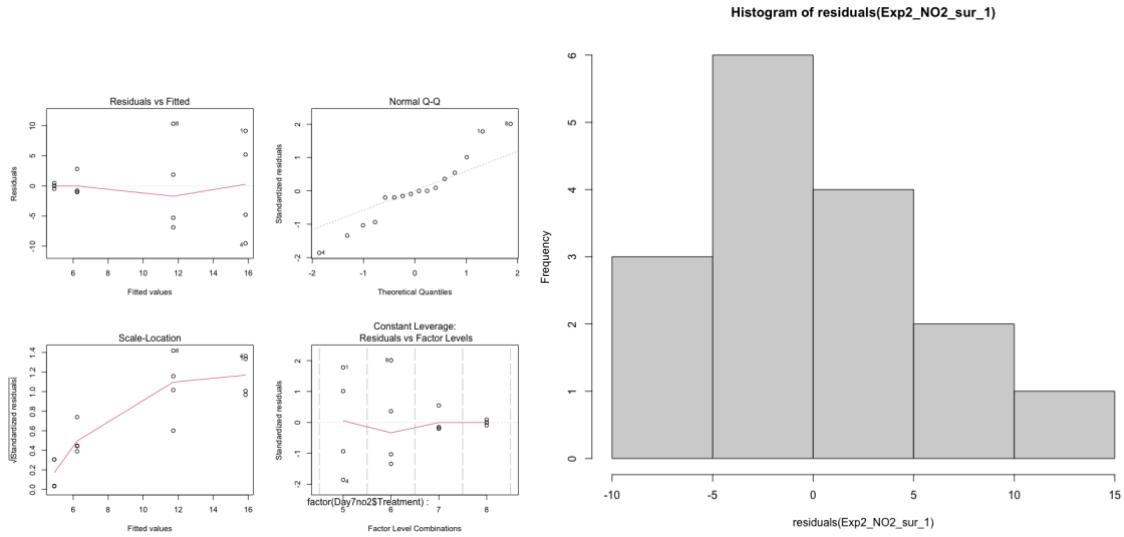

Figure C.11. Fit of the model for Nutrient left in beaker $No_2^-$of the Seaweed Experiment II

Table C.12. Results of Seaweed Experiment II: Nutrients in beaker No_3^-Day 7. The stars in the right column denote the significance codes between: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. The density treatments 5-8 used in this experiment. Respectively, they denote 100% (RAS), 200 % (RAS), 300% (RAS) and 100 % (Artificial seawater) biomass.

LM(FORMULA = NO_3^-~ TREATMENT)					
RESIDUALS:					
	Min	1Q	Median	3Q	Max
	-5187.9	-498.1	-104.8	698.5	3046.1
COEFFICIENTS:					
	Estimate	Std. Error	t value	Pr(>|t|)	Significance level
(INTERCEPT)	15659	1034	15.15	<0.001	***
TREATMENT 6	-9006	1462	-6.16	<0.001	***
TREATMENT 7	-8476	1462	-5.80	<0.001	***
TREATMETN 8	-7618	1462	-5.21	<0.001	***
OTHER					
ADJUSTED R-SQUARED:	0.76				
P-VALUE:	<0.001				

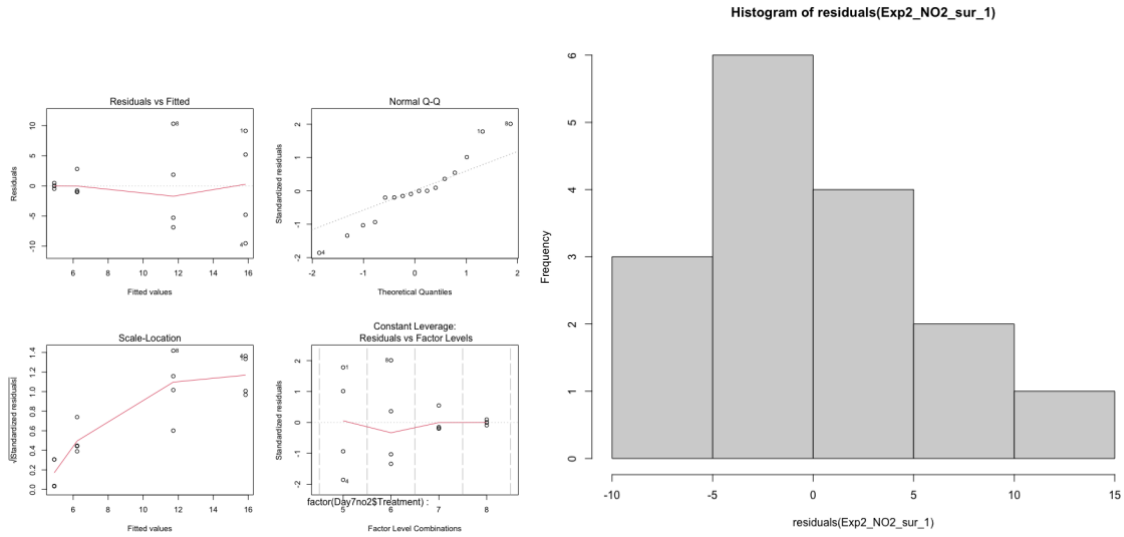

Figure C.12. Fit of the model for Nutrient left in beaker $No_3^-$of the Seaweed Experiment II

Table C.13. Results of Seaweed Experiment II: Nutrients in beaker PO_4^{3-} Day 7. The stars in the right column denote the significance codes between: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. The density treatments 5-8 used in this experiment. Respectively, they denote 100% (RAS), 200 % (RAS), 300% (RAS) and 100 % (Artificial seawater) biomass.

LM(FORMULA = PO_4^{3-} ~ TREATMENT)					
RESIDUALS:					
	Min	1Q	Median	3Q	Max
	-44.86	-2.17	-0.51	2.75	44.86
COEFFICIENTS:					
	Estimate	Std. Error	t value	Pr(>|t|)	Significance level
(INTERCEPT)	146.72	14.46	10.15	<0.001	***
TREATMENT 6	-126.31	17.71	-7.13	<0.001	***
TREATMENT 7	-134.91	17.71	-7.62	<0.001	***
TREATMETN 8	-143.23	17.71	-8.01	<0.001	***
OTHER					
ADJUSTED R-SQUARED:	0.85				
P-VALUE:	<0.001				

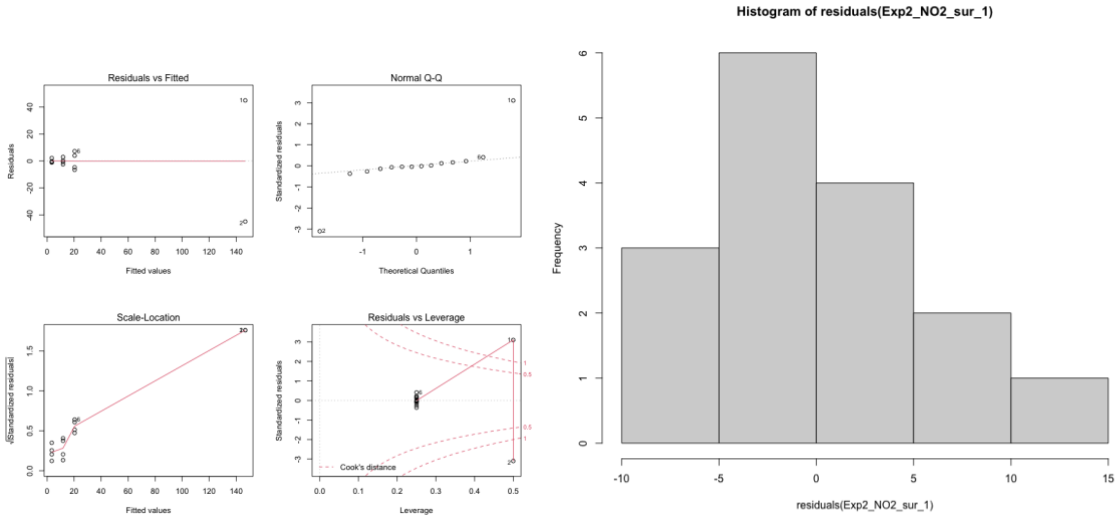

Figure C.13. Fit of the model for Nutrient left in beaker $PO_4^{3-}$of the Seaweed Experiment II

10. Appendix E: The model

Table E.1. In- and output for the snails and seaweed for the size allocation of 1:1

SIZE ALLOCATION 1:1					
INPUT DAYS	*Year*	*Input*	*Harvest days*	*Year*	*Output snails*
0	1	175000	0-157	1	33688
157	1	43750	0-313	1	33688
313	1	43750	0-470	2	33688
470	2	43750	0-627	2	33688
627	2	43750	157-784	3	33688
784	3	43750	313-940	3	33688
940	3	43750	470-1097	4	33688
1097	3	43750	627-1254	4	33688
1254	4	43750	784-1411	4	33688
			940-1567	5	33688
TOTAL		**525000**	1097-1724	5	33688
			1254-1881	5	33688
			TOTAL		**404256**

Table E.2. In- and output for the snails and seaweed for the size allocation of 1:3

SIZE ALLOCATION 1:3					
INPUT DAYS	*Year*	*Input*	*Harvest days*	*Year*	*Snails*
0	1	87500	0-157	1	16844
157	1	21875	0-313	1	16844
313	1	21875	0-470	2	16844
470	2	21875	0-627	2	16844
627	2	21875	157-784	3	16844
784	3	21875	313-940	3	16844
940	3	21875	470-1097	4	16844
1097	3	21875	627-1254	4	16844
1254	4	21875	784-1411	4	16844
			940-1567	5	16844
TOTAL		**262500**	1097-1724	5	16844
			1254-1881	5	16844
			TOTAL		**202125**

Table E.3. In- and output for the snails and seaweed for the size allocation of 4.5:5.5

SIZE ALLOCATION: 4.5:5.5					
INPUT DAYS	*Year*	*Input snails*	*Harvest days*	*Year*	*Output snails*
0	1	157500	0-157	1	30319
157	1	39375	0-313	1	30319
313	1	39375	0-470	2	30319
470	2	39375	0-627	2	30319
627	2	39375	157-784	3	30319
784	3	39375	313-940	3	30319
940	3	39375	470-1097	4	30319
1097	3	39375	627-1254	4	30319
1254	4	39375	784-1411	4	30319
			940-1567	5	30319
TOTAL		**472500**	1097-1724	5	30319
			1254-1881	5	30319
			TOTAL		**363825**

Table E.4. The net growth of seaweed for the size allocation of 1:1 over a period of five years.

SIZE ALLOCATION 1:1								
YEAR	*Days*	*Start biomass (kg FW)*	*Treatment*	*Growth rate*	*Grazing rate snails (kg t_i^{-1})*	*Total biomass (kg FW)*	*Net biomass (kg FW)*	*Surplus (kg FW)*
1	7	1120	1	1.087403	40.72	2013.50		
1	7		1	1.025255	40.72	2397.59		
1	7		1	1.017770	40.72	2712.20		
1	344		1	1.001319	2000.99	4267.78	3841.00	1717.85
2	365		1	1.001319	2123.15	2778.98	2501.08	377.93
3	365		1	1.001319	2123.15	611.38	550.25	-1572.90
3	7	1120	1	1.087403	40.72	2013.50		
3	7		1	1.025255	40.72	2397.59		
3	7		1	1.017770	40.72	2712.20		
3	344		1	1.001319	2000.99	4267.78	3841.00	1717.85
4	365		1	1.001319	2123.15	2778.98	2501.08	377.93
5	365		1	1.001319	2123.15	611.38	550.25	-1572.90
5	7	1120	1	1.087403	40.72	2013.50		
5	7		1	1.025255	40.72	2397.59		
5	7		1	1.017770	40.72	2712.20		
5	344		1	1.001319	2000.99	4267.78	3841.00	1717.85

Table E.5. The net growth of seaweed for the size allocation of 1:3 over a period of five years.

SIZE ALLOCATION 1:3								
YEAR	*Day*	*Start biomass (kg FW)*	*Treatment*	*Growth rate*	*Grazing rate snails (kg t_i^{-1})*	*Total biomass (kg FW)*	*Net biomass (kg FW)*	*Surplus*
1	7	1680	1	1.087402667	20.35895944	3020.244051		
1	7		1	1.025255333	20.35895944	3596.385994		
1	7		1	1.017769667	20.35895944	4068.298585		
1	344		1	1.0013187	1000.497435	6401.669615	5761.502654	4699.93
2	365		1	1.0013187	1061.574314	7603.097109	6842.787398	5781.213085
3	365		1	1.0013187	1061.574314	9352.296739	8417.067065	7355.492751
4	365		1	1.0013187	1061.574314	11899.01667	10709.115	9647.54069
5	365		1	1.0013187	1061.574314	15606.87385	14046.18647	12984.61215

Table E.6. The net growth of seaweed for the size allocation of 4.5:5.5 over a period of five years.

SIZE ALLOCATION 4.5:5.5								
YEAR	*Day*	*Start biomass (kg FW)*	*Treatment*	*Growth rate*	*Grazing rate snails (kg t_i^{-1})*	*Total biomass (kg FW)*	*Net biomass (kg FW)*	*Surplus*
1	7	1232	1	1.087403	36.65	2214.85		
1	7		1	1.025255	36.65	2637.35		
1	7		1	1.017770	36.65	2983.42		
1	344		1	1.001319	1800.90	4694.56	4225.10	2314.27
2	365		1	1.001319	1910.83	3743.80	3369.42	1458.59
3	365		1	1.001319	1910.83	2359.57	2123.61	212.78
4	365		1	1.001319	1910.83	344.21	309.79	-1601.05
4	7	1232	1	1.087403	36.65	2214.85		
4	7		1	1.025255	36.65	2637.35		
4	7		1	1.017770	36.65	2983.42		
4	344		1	1.001319	1800.90	4694.56	4225.10	2314.27
5	365		1	1.001319	1910.83	3743.80	3369.42	1458.59

Table E.7. The total in- and output for all robustness scenarios

5 % INCREASEGRAZING RATE				
	Snails (ind)		Seaweed (kg)	
YEAR	Input	Ouput	Input	Ouput
1	210000	53900	1344	
2	70000	53900		
3	105000	53900		
4	35000	80850		560
5		80850	1344	2826
TOTAL	**420000**	**323400**	**2688**	**3386**
5 % DECREASE GRAZING RATE				
	Snails (ind)		Seaweed (kg)	
YEAR	Input	Ouput	Input	Ouput
1	210000	53900	1344	
2	70000	53900		
3	105000	53900		
4	35000	80850		
5		80850		1097
TOTAL	**420000**	**323400**	**1344**	**1097**
5 % INCREASE GROWTH RATE SEAWEED				
	Snails (ind)		Seaweed (kg)	
YEAR	Input	Ouput	Input	Ouput
1	210000	53900	1344	
2	70000	53900		
3	105000	53900		
4	35000	80850		
5		80850		2297
TOTAL	**420000**	**323400**	**1344**	**2297**
5 % DECREASE GROWTH RATE SEAWEED				
	Snails (ind)		Seaweed (kg)	
YEAR	Input	Ouput	Input	Ouput
1	210000	53900	1344	
2	70000	53900		
3	105000	53900		1177
4	35000	80850	1344	
5		80850		2023
TOTAL	**420000**	**323400**	**2688**	**3199**
5 % INCREASE MORTALITY SEAWEED				
	Snails (ind)		Seaweed (kg)	
YEAR	Input	Ouput	Input	Ouput
1	210000	53900	1344	

2	70000	53900		
3	105000	53900		218
4	35000	80850	1344	
5		80850		1393
TOTAL	**420000**	**323400**	**2688**	**1611**

5 % DECREASE MORTALITY SEAWEED

	Snails (ind)		Seaweed (kg)	
YEAR	Input	Ouput	Input	Ouput
1	210000	53900	1344	
2	70000	53900		
3	105000	53900		
4	35000	80850		
5		80850		3179
TOTAL	**420000**	**323400**	**1344**	**3179**

5 % INCREASE MORTALITY SNAILS

	Snails (ind)		Seaweed (kg)	
YEAR	Input	Ouput	Input	Ouput
1	210000	50400	1344	
2	70000	50400		
3	105000	50400		
4	35000	75600		
5		75600		1405
TOTAL	**420000**	**302400**	**1344**	**1405**

10 % DECREASE MORTALITY SNAILS

	Snails (ind)		Seaweed (kg)	
YEAR	Input	Ouput	Input	Ouput
1	210000	57400	1344	
2	70000	57400		
3	105000	57400		989
4	35000	86100	1344	
5		86100		1997
TOTAL	**420000**	**365 400**	**2688**	**2987**

Table E.8: Total seaweed consumption for robustness scenarios.

GRAZING + 5%

YEAR	Growth specification	added biomass	Treatment	Growth rate	Grazing rate snails (kg / x)	Total biomass	Net biomass (after mortality)	Surplus
1	7	1344	1	1.08740267	34.20	2416.20		
1	7		1	1.02525533	34.20	2877.11		
1	7		1	1.01776967	34.20	3254.64		
1	344		1	1.0013187	1680.84	5121.34	4609.20	2825.76
2	365		1	1.0013187	1783.44	4571.24	4114.12	2330.67
3	365		1	1.0013187	1783.44	3770.34	3393.31	1609.86
4	365		1	1.0013187	1783.44	2604.28	2343.85	560.41
5	365		1	1.0013187	1783.44	906.57	815.92	-967.53
5	7	1344	1	1.08740267	34.20	2416.20		
5	7		1	1.02525533	34.20	2877.11		
5	7		1	1.01776967	34.20	3254.64		
5	344		1	1.0013187	1680.84	5121.34	4609.20	2825.76

GRAZING – 5%

YEAR	Growth specification	added biomass	Treatment	Growth rate	Grazing rate snails (kg / x)	Total biomass	Net biomass (after mortality)	Surplus
1	7	1344	1	1.08740267	30.95	2416.20		
1	7		1	1.02525533	30.95	2877.11		
1	7		1	1.01776967	30.95	3254.64		
1	344		1	1.0013187	1520.76	5121.34	4609.20	2995.61
2	365		1	1.0013187	1613.59	4846.01	4361.41	2747.82
3	365		1	1.0013187	1613.59	4445.16	4000.64	2387.05
4	365		1	1.0013187	1613.59	3861.54	3475.39	1861.79
5	365		1	1.0013187	1613.59	3011.83	2710.65	1097.06

GROWTH SEAWEED + 5%

YEAR	Growth specification	added biomass	Treatment	altered growth rate	Grazing rate snails (kg / x)	Total biomass	Net biomass (after mortality)	Surplus
1	7	1344	1	1.0917728	32.57	2484.99		
1	7		1	1.0265181	32.57	2984.64		
1	7		1	1.01865815	32.57	3396.96		
1	344		1	1.00138464	1600.80	5467.75	4920.97	3222.45
2	365		1	1.00138464	1698.52	5339.78	4805.80	3107.29
3	365		1	1.00138464	1698.52	5148.94	4634.05	2935.53
4	365		1	1.00138464	1698.52	4864.34	4377.90	2679.39
5	365		1	1.00138464	1698.52	4439.89	3995.90	2297.38

GROWTH SEAWEED – 5%

YEAR	Growth specification	added biomass	Treatment	altered growth rate	Grazing rate snails (kg / x)	Total biomass	Net biomass (after mortality)	Surplus
1	7	1344	1	1.08303253	32.57	2349.04		

1	7		1	1.02399257	32.57	2773.11		
1	7		1	1.01688118	32.57	3117.88		
1	344		1	1.00125277	1600.80	4796.25	4316.62	2618.10
2	365		1	1.00125277	1698.52	4134.73	3721.26	2022.74
3	365		1	1.00125277	1698.52	3194.49	2875.04	1176.52
4	365		1	1.00125277	1698.52	1858.06	1672.26	-26.26
4	7	1344	1	1.08303253	32.57	2349.04		
4	7		1	1.02399257	32.57	2773.11		
4	7		1	1.01688118	32.57	3117.88		
4	344		1	1.00125277	1600.80	4796.25	4316.62	2618.10
5	365		1	1.00125277	1698.52	4134.73	3721.26	2022.74

MORTALITY SEAWEED + 5%

YEAR	Growth specification	added biomass	Treatment	Growth rate	Grazing rate snails (kg / x)	Total biomass	Net biomass (after mortality)	Surplus
1	7	1344	1	1.08740267	32.57	2416.20		
1	7		1	1.02525533	32.57	2877.11		
1	7		1	1.01776967	32.57	3254.64		
1	344		1	1.0013187	1600.80	5121.34	4353.14	2654.62
2	365		1	1.0013187	1698.52	4294.39	3091.96	1393.44
3	365		1	1.0013187	1698.52	2254.17	1916.05	217.53
4	365		1	1.0013187	1698.52	351.90	299.11	-1399.41
4	7	1344	1	1.08740267	32.57	2416.20		
4	7		1	1.02525533	32.57	2877.11		
4	7		1	1.01776967	32.57	3254.64		
4	344		1	1.0013187	1600.80	5121.34	4353.14	988.67
5	365		1	1.0013187	1698.52	4294.39	3650.23	1393.44

MORTALITY SEAWEED – 5%

YEAR	Growth specification	added biomass	Treatment	Growth rate	Grazing rate snails (kg / x)	Total biomass	Net biomass (after mortality)	Surplus
1	7	1344	1	1.08740267	32.57	2416.20		
1	7		1	1.02525533	32.57	2877.11		
1	7		1	1.01776967	32.57	3254.64		
1	344		1	1.0013187	1600.80	5121.34	4865.27	3166.75
2	365		1	1.0013187	1698.52	5122.87	4866.72	3168.20
3	365		1	1.0013187	1698.52	5125.22	4868.96	3170.44
4	365		1	1.0013187	1698.52	5128.84	4872.40	3173.88
5	365		1	1.0013187	1698.52	5134.40	4877.68	3179.16

MORTALITY SNAILS + 5%

YEAR	Growth specification	added biomass	Treatment	Growth rate	Grazing rate snails (kg / x)	Total biomass	Net biomass (after mortality)	Surplus
1	7	1344	1	1.08740267	30.46	2416.20		
1	7		1	1.02525533	30.46	2877.11		
1	7		1	1.01776967	30.46	3254.64		
1	344		1	1.0013187	1496.85	5121.34	4609.20	3020.98

2	365		1	1.0013187	1588.23	4887.05	4398.34	2810.12
3	365		1	1.0013187	1588.23	4545.94	4091.35	2503.12
4	365		1	1.0013187	1588.23	4049.31	3644.38	2056.16
5	365		1	1.0013187	1588.23	3326.26	2993.63	1405.40

MORTALITY SNAILS – 10%

YEAR	Growth specification	added biomass	Treatment	Growth rate	Grazing rate snails (kg / x)	Total biomass	Net biomass (after mortality)	Surplus
1	7	1344	1	1.08740267	36.80	2416.20		
1	7		1	1.02525533	36.80	2877.11		
1	7		1	1.01776967	36.80	3254.64		
1	344		1	1.0013187	1808.69	5121.34	4609.20	2690.10
2	365		1	1.0013187	1919.11	4531.78	3916.60	1997.50
3	365		1	1.0013187	1919.11	3231.36	2908.23	989.12
4	365		1	1.0013187	1919.11	1600.10	1440.09	-479.01
5	365		1	1.0013187	36.80	2416.20		
5	7	1344	1	1.08740267	36.80	2877.11		
5	7		1	1.02525533	36.80	3254.64		
5	7		1	1.01776967	1808.69	5121.34	4609.20	2690.10
5	344		1	1.0013187	1919.11	4351.78	3916.60	1997.50

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