Master's thesis

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Exploring the Potential for Hydroponic Plant Growth in Water from Atlantic Salmon Recirculating Aquaculture Systems (Aquaponics)

Master's thesis in Food Science, Technology and Sustainability
Supervisor: Jørgen Lerfall
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Trondheim, May 2022

Astrid Kristine Tennøy

Abstract

The global population is constantly growing, and the UN predicts the population to reach 9,8 billion in 2050. A significant increase in food production to secure this growth is problematic due to challenges with scarce freshwater resources, lack of agricultural land, pollution, and eutrophication. New methods for sustainable food production must be explored to secure healthy and safe food for both people and the environment. Norway has a large aquaculture industry, which is considered important in the production of sustainable animal protein. Recirculating aquaculture systems (RAS) provide full control of all inputs and outputs of the aquaculture system, allowing for an increased production and collection of waste products. The waste products consist of valuable nutrients such as nitrogen and phosphorus, which can be further utilized. Combining RAS and hydroponic growth of vegetables can create a more sustainable food system through aquaponics, where the waste products from fish are used as nutrients for plants.

To explore the potential of plant growth in RAS water from Atlantic salmon production, a literature review was performed, accompanied by a plant experiment. The main objective was to see whether lettuce (*Lactuca sativa)* production could be used as a filtering method to remove nitrogen and phosphorus compounds from water in RAS production of salmon smolt, to replace denitrification and de-phosphorus processes. Water was extracted from two locations in RAS; after the biofilter and from the plate separator. The results indicate that lettuce production can be used as part of the filtering process to remove nitrogen and phosphorus compounds from the water, but removal rates were too low to replace denitrification and de-phosphorus processes. Yet, plants grew throughout the experiment, similarly to soil plants, and there is a potential for plant growth. No significant differences were seen for plants in the two different water types. Adding nutrients to complement the RAS system, through decoupled systems can improve plant growth and contribute to a circular economy of water and nutrients available in RAS.

This research contributes to fill an information gap regarding aquaponics in combination with Atlantic salmon production in RAS. The industry is now researching how to integrate plant sections in RAS and create more sustainable food productions. Most data found on aquaponics is related to warm-water species and there are few scientific publications on cold-water species such as Atlantic salmon.

Sammendrag

Den globale befolkningen vokser stadig, og FN spår at den vil nå 9,8 milliarder i 2050. For å sikre denne veksten, må en betydelig økning i matproduksjonen til. Dette er problematisk på grunn av utfordringer knyttet til knappe ferskvannsressurser, mangel på egnet jordbruksareal, og forurensning av miljøet. Nye metoder for bærekraftig matproduksjon må utforskes for å sikre sunn og trygg mat for mennesker og miljø. Norge har en stor havbruksnæring, som anses viktig i produksjon av bærekraftig animalsk protein. Resirkulerende akvakultursystemer (RAS) gir full kontroll over alle parameter inn og ut av systemene, noe som gir mulighet for økt produksjon og oppsamling av avfallsprodukter. Avfallsproduktene består av verdifulle næringsstoffer som nitrogen og fosfor, som kan utnyttes videre. Gjennom å kombinere RAS og hydroponisk vekst av grønnsaker kan man skape et mer bærekraftig matsystem gjennom akvaponi, hvor avfallsprodukter fra fisk brukes som næringsstoffer for planter.

For å utforske potensialet for plantevekst i RAS-vann fra produksjon av atlantisk laks, ble det utført en litteraturstudie, samt et planteeksperiment. Hovedmålet var å se om salatproduksjon (*Lactuca sativa*) kunne brukes som filtreringsmetode for å fjerne nitrogen- og fosforforbindelser fra produksjonsvann i RAS med laksesmolt, for å erstatte denitrifikasjons- og de-fosforprosesser. Det ble hentet vann fra to steder i RAS; etter biofilteret og fra plateseparatoren. Resultatene indikerer at salatproduksjon kan brukes som en del av filtreringsprosessen for å fjerne nitrogen- og fosforforbindelser fra vannet, men filtreringen var for lav til å erstatte denitrifikasjons- og de-fosforprosesser. Plantene vokste likevel gjennom hele forsøket, på lik linje med jordplanter, og et potensiale for plantevekst ble observert. Det ble ikke observert signifikante forskjeller for planter i de to ulike vanntypene. Ved bruk av de-koblede systemer, kan man tilsette næringsstoffer og forbedre næringsløsningen i vannet fra RAS. Dette kan forbedre planteveksten og bidra til en sirkulær økonomi av vann og næringsstoffer tilgjengelig i RAS.

Denne forskningen bidrar til å fylle et behov for informasjon om akvaponi i kombinasjon med produksjon av atlantisk laks i RAS. Næringen forsker nå på hvordan man kan innlemme planteseksjoner i RAS og skape en mer bærekraftig matproduksjon. Mye av litteraturen på akvaponi er relatert til varmtvannsarter, og det er få vitenskapelige publikasjoner om kaldtvannsarter som atlantisk laks.

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Abbreviations

AC	Ash content
AOM	Ammonia-oxidizing microorganisms
CI	Confidence interval
CV	Cultivar
CP	Control plants
DGR	Daily Growth Rate
DO	Dissolved oxygen
DNS	Denitrification system
DW	Dry weight
DWS	Deep water system
EC	Electrical conductivity
EGSB	Expanded granular sludge bed
EPS	Expanded polystyrene sheets
EQ	Equation
FAO	Food and Agriculture Organization of the United Nations
FS	Food systems
FW	Fresh weight
GM	Growth media
GM1	Growth media 1
GM2	Growth media 2
LCA	Life cycle analysis
HSD	Tukey's honest significance difference
MLH	Multiple limitation hypothesis
MNH	Midt-Norsk Havbruk AS (MNH)
N	Number of samples
N_2	Nitrogen gas
NFT	Nutrient film technique
NH_3	Ammonia
NH_4^+	Ammonium
H_2S	Hydrogen sulfide
NOB	Nitrite-oxidizing bacteria
NTNU	Norwegian University of Science and Technology
NO_3^-	Nitrate
NO_2^-	Nitrite
OWA	One-way ANOVA
RAS	Recirculating aquaculture system
SD	Standard deviation
SDG	Sustainable development goals
SFS	Sustainable food system
SS	Suspended solids
TAN	Total ammonia nitrogen
TSS	Total suspended solids
UASB	Up-flow anaerobic sludge blanket reactor
$\bar{x}$	Mean average

Introduction

The global population is constantly growing, and the UN predicts the population to reach 9,8 billion in 2050 (UN, 2017). To support this rapid population growth, food production needs to be increased by 70 percent (FAO, 2009; Godfray, 2010). Scarce freshwater resources, lack of agricultural land, pollution, and eutrophication are some of the challenges connected to food production (FAO, 2017; Ritchie, 2017; Smith & Schindler, 2009; Springmann et al., 2018). To maintain an elevated production rate, more sustainable food systems must be implemented. The production of salmon is one of the most sustainable ways to produce animal protein for human consumption (Graber & Junge, 2009; Palm et al., 2019). In 2021, Norway's export of Atlantic salmon surpassed 120 billion NOK (Norges Sjømatråd, 2022). However, several challenges are linked to the traditional sea-based aquaculture, such as sea lice, fish escapes and pollution. Land-based solutions are therefore becoming increasingly popular, and recirculating aquaculture systems (RAS) give full control of parameters in and out of the production (Lekang et al., 2016). Waste products from RAS contain considerable amounts of nutrients such as nitrogen (N) and phosphorus (P), which can be further utilized. Combining RAS and hydroponic growth of vegetables can create more sustainable food systems through aquaponics, where the waste products from fish are used as nutrients for plants (Espinal & Matulić, 2019; Goddek, Joyce, Kotzen, et al., 2019).

1.1 Sustainable Food Systems

Sustainable food systems (SFS) are at the heart of the United Nations Sustainable Development Goals (SDG) (Figure 1-A), which focus on the need to make changes within agriculture and food systems, to decrease world hunger, as well as to achieve food security and nutrition (Nguyen, 2018). Food systems (FS) are systems that include all key actors and their interlinked value-adding activities in all processes from production to disposal of food products (von Braun et al., 2020). FAO states that an SFS should provide food security and nutrient for everyone now and in the future, and generate positive values related to the three pillars of sustainability; economic, social, and environmental (Figure 1-B) (Nguyen, 2018).

Figure 1 – Figure A show the sustainable development goals, given by the UN (2022). Figure B show the three pillars of sustainability by Smart Cities (2021)

The 'Farm to Fork'- strategy of the UN aims to reduce excess fertilization (a 20% reduction by 2030) and increase organic farming, for Europe to become the first climate-neutral continent by 2050. To achieve this, sustainable nutrient management of N and P is important (European Commission, 2020). The planetary boundaries (Figure 2) define environmental limits humanity safely can operate within with regards to scarce resources (Rockström et al., 2009). The biochemical flow boundaries are more limiting to food supply than climate change (Steffen et al., 2015). Both N and P is currently beyond the zone of uncertainty, meaning at high risk. Nutrient recycling, dietary changes and waste prevention are considered necessary to transform todays production (Goddek, Joyce, Kotzen, et al., 2019). Plants do not efficiently absorb all nutrients utilized in agriculture, which causes an excess leading to climate impacts through air, soil, and water pollution. N and P are of special importance, as it leads to the eutrophication of freshwater resources and affects biodiversity (Smith & Schindler, 2009). To ensure sustainable food production farmers, fishers, and aquaculture producers must therefore transform their production methods to make the best use of the natural resources (European Commission, 2020)

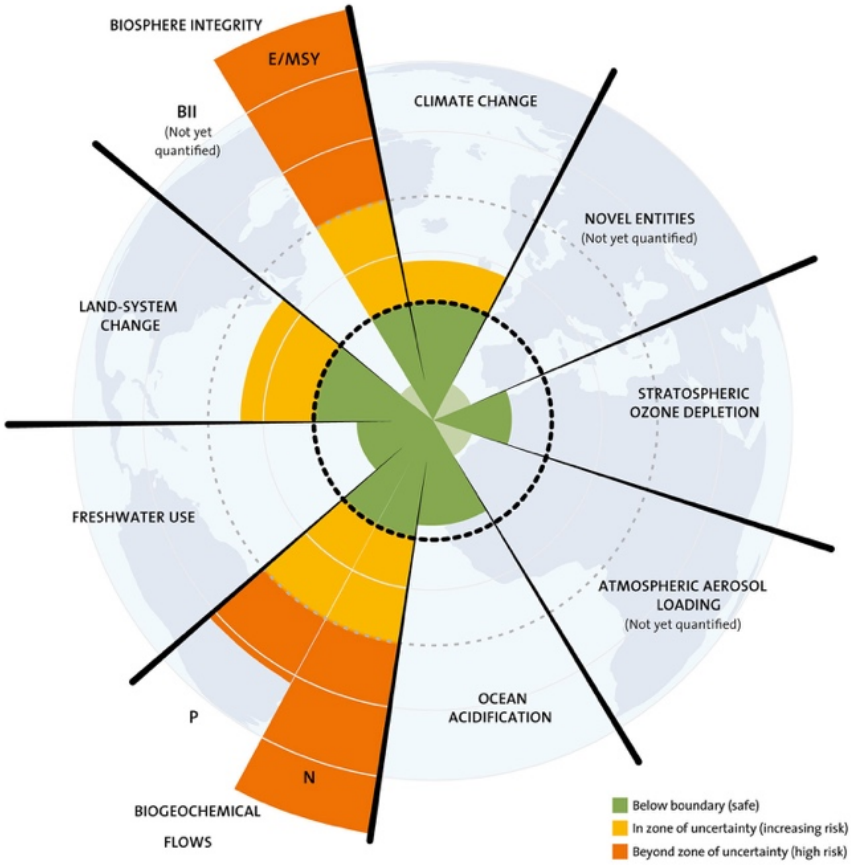

Figure 2 - Planetary boundaries as presented by Steffen et al. (2015). The figure shows the status of the boundaries, where the green zone is 'below boundary' meaning safe operating space, yellow is 'in zone of uncertainty' meaning an increased risk and orange is 'beyond zone of uncertainty' meaning high risk. The nutrients nitrogen and phosphorus are both at high risk.

1.2 Research Questions and Objectives

The main objective of this thesis is to research whether lettuce (*Lactuca sativa)* production can be used as a filtering method to remove nitrogen and phosphorus compounds from water in RAS production of Atlantic salmon smolt, to replace denitrification and de-phosphorus processes. Secondary objectives were to build and perform a hydroponic experiment to research the filtering effect (nutrient uptake) of *Lactuca sativa,* to study the potential for plant growth in RAS water and explore where to integrate hydroponics in the process. The goal is to recycle valuable nutrients and explore a more sustainable food system. The study focuses solemnly on Norwegian land-based smolt production, as the later phase in the salmon lifecycle is in saltwater. Economic aspects are not considered in this thesis.

2 Background

Traditional agriculture practices put strains on the environment in forms of climate change, pollution and deforestation, and leads to a general degrading of the environment (Sharma et al., 2018; Springmann et al., 2018; Wanza, 2018). At the same time, there are vast areas along the coasts that can be used for aquaculture (FAO, 2018b, 2019). The State of World Fisheries and Aquaculture 2020 report by FAO states that the growth of the fishery and aquaculture sector is important to provide enough food, nutrition and employment for the world population. Sjømat Norge (2018) states that the Norwegian aquaculture sector should be increased to reach a production capacity of 5 million tons by 2050. This will generate significant amounts of waste products. The use of land-based aquaculture solutions is increasing and provide opportunities to collect waste products and further utilize them (Lekang et al., 2016). A more SFS can be created by connecting a plant section to this aquaculture production and recycle the nutrients. This chapter will further explain different aspects related to the concept of aquaponics.

2.1 Aquaculture

Aquaculture is the cultivation of aquatic species in controlled environments, mainly for human consumption. The Global Seafood Alliance predicts that by 2030, 62% of the global consumption of seafood will come from aquaculture. Today the number is 50% (Global Seafood Alliance, 2019). Aquaculture is a more efficient way of producing protein and which use less resources than production of other agriculture animals (Graber & Junge, 2009; Palm et al., 2019). Since 1970 aquaculture production has increased by 7,5% annually, and this growth is considered crucial for global food security (FAO, 2020). To keep up with the growing population, the production must be increased by 75 million tons compared to the production in 2010 (Waite et al., 2014). This intensification in the aquaculture industry leads to environmental challenges that need to be addressed (section 2.1.2) (FAO, 2020). Norway is the world's largest producer of salmonids (salmon, trout, etc.), followed by Chile. Salmonid production is the most technically advanced and profitable aquaculture industry globally (FAO, 2020). FAO has put focus on sustainable development strategies, implementing technologies and policies to promote sustainability. Integrated aquaculture and aquaponics are examples of sustainable production methods (FAO, 2020).

2.1.1 Atlantic Salmon

Atlantic salmon (*Salmo Salar*) is an anadromous species in the salmonid family. Anadromous means that the fish is born in rivers, migrates to the ocean to feed, and returns to the river again to spawn (Fleming, 1996). Norway has had a large aquaculture sector producing Atlantic salmon since the 1970s. The fish is hatched and reared in freshwater in land-based facilities until they are ready to smoltify (8-18 months) (Hansen, 2019). When the fish smoltifies, it goes through physiological changes preparing it to migrate from freshwater to seawater. Traditionally the smolt has been transferred to sea-based production units (Gross, 1998). The industry is now changing towards more land-based or closed cages for post-smolt and full-grown salmon production.

2.1.2 Land-Based Aquaculture and RAS-Systems

Due to environmental challenges, issues with salmon lice, and fish escapes, more salmon producers are increasingly moving the production to land-based facilities. Land-based facilities implies higher control on all parameters going in and out of the system (Lekang et al., 2016). Scarce freshwater resources, land area use and emission of waste products are some of the challenges that can be addressed through land-based aquaculture (Dahle et al., 2022).

European aquaculture-producing countries are promoting RAS systems, and consider this a possible solution to further increase the growth of the sector (Badiola et al., 2012). RAS optimizes the water use, recycling 90-99% of the water through advanced filtration processes (Badiola et al., 2012). The technology allow for high-intensity aquaculture, while simultaneously reducing the nutrient discharges (e.g. N and P) and environmental impacts (Badiola et al., 2012). Solid waste is collected, reducing the impact of aquaculture compared to flow-through systems (Dauda et al., 2019; Pedersen et al., 2008). Other advantages are lower impact on habitat destruction, eutrophication and biotic depletion as well as fewer disease outbreaks and parasite transmission (Ahmed & Turchini, 2021). Areas not previously suitable for traditional food production can also be used for production in RAS (Badiola et al., 2012).

The systems are advanced technology-biology interacting systems, that require monitoring and educated and experienced personnel (Lekang, 2007).

2.1.3 Waste Products from Land-Based Aquaculture

The waste product from aquaculture production mainly consists of uneaten feed and fish feces, which is high in nutrients such as N and P compounds (Cripps & Bergheim, 2000; Ebeling & Timmons, 2012). The composition may vary during the production cycle. The type of feed, growth stage of the fish, feeding technology, and other operating factors affect the quality of the waste product (Cabell et al., 2019). The feeding ratio is of high importance for the amount of sludge and nutrient content (Skarra, 2020).

Waste products from land-based aquaculture production consist of two parts; solid waste and wastewater. It starts off as a sludge, which through mechanical filtration, is separated into the different phases (Ebeling & Timmons, 2012). Up to 50% of the waste product is feed which is more easily removed than feces (Ytrestøyl et al., 2016). However, both feces and undigested feed dissolve and pass through the filters. Most water-soluble nutrients are released, while those bound to particles are captured (Cabell et al., 2019). In Norway, official requirements are set to ensure that the discharge water is treated (filtered and cleaned), and levels of nutrients and particles are similar to the levels of municipal wastewater. This to comply with the Pollution Control Act (Biogass Oslofjord, 2018), to avoid overload of nutrients in coastal areas (Brod, 2021). Therefore, new and modernized facilities in Norway, are required to treat the wastewater and reduce the amount of suspended matter (particles>45µm) by 70% or more (Biogass Oslofjord, 2018).

2.2 Hydroponics

The production of greens and vegetables in nutrient-rich water, without the use of soil, is called hydroponics, and is defined as *"the process of growing plants in sand, gravel, or liquid, with added nutrients but without soil"*(Lexico, 2022). Meaning that the roots are in direct contact with nutrient-rich water that provides the right nutrients and promote growth. Hydroponics is becoming increasingly popular amongst food producers, and who establish indoor systems to avoid problems with disease, pesticide use, fertilizers, and limiting water use. The method enables food production to be more sustainable and safe, and includes water and nutrient recycling (Cifuentes-Torres et al., 2021).

The production method provides countless possibilities and constellations for plant production, as it does not depend on soil and heavy constructions (Resh, 2015). The systems can be

structured as horizontal and vertical modules, enabling growth all year round and utilizing less water (Resh, 2015). With soil plants, much of the irrigation water is lost due to drainage or evaporation. Hydroponic systems can be closed to minimize evaporation. This also means systems can be set up in areas with poor environmental conditions for plant growth, creating new areas for food production (Baras, 2018). With optimal combinations of nutrients, light and temperature, hydroponics can give a faster growth than traditional agriculture (Resh, 2015). To ensure optimal growth, all necessary nutrients must be added to the aqueous solution, and the right amount of high-quality fertilizer is important. If the plants are exposed to a lack of or abundance of nutrients, it will quickly affect the plants. Hydroponic plants are extra sensitive since the roots are in direct contact with the water (Sanchez, 2020).

The optimal pH for hydroponic systems is 5,0 to 7,0 (Sanchez, 2020). If the pH is too low or too high, plants cannot absorb the nutrients, which may lead to deficiencies. The different nutrients need certain pH values for the plant to be able to absorb them. Plants may also require acidic or alkaline conditions, depending on the species. Compared to soil plants, hydroponic plants have different needs in terms of pH. This is because they cannot benefit from organic matters, microorganisms, and minerals that are available in the soil and that could help regulate the pH levels. Water temperatures are recommended to be stable at around 18-26°C for hydroponic plants (Jenco, 2019). Oxygen levels should be above 4-5 mg/l, to prevent nutrient deficiencies related to low uptake performance by the root system (Maucieri et al., 2019)

2.2.1 Deep Water Culture

Deep Water Culture (DWC) is one of the most used methods for growth in commercial hydroponic systems (Majid et al., 2021; Valdez, 2017). In a deep-water culture, the roots are always immersed in nutrient-rich water and air stones are used to provide air for the plants. Simple systems with boxes or buckets and net pots can be used and work well for larger plants such as cucumber and tomato. Large-scale systems often structure floating rafts, which are much used in the production of green leafy plants (Eck et al., 2019; Resh, 2022). Here the sprouts are placed in net pots on a floating raft, at one end of a long channel filled with 10-30 cm nutrient-rich water. Once the plants are fully grown, they are harvested at the other end of the channel (Sharma et al., 2018). A slow water stream can be included to continuously replenish the plants with nutrients (Maucieri et al., 2019). Oxygen and nutrient concentrations

must be monitored, as well as salinity and pH (Domingues et al., 2012). The system requires low management and minimizes costs (Maucieri et al., 2019).

2.3 Aquaponics

Aquaponics is a technique that combines aquaculture with the hydroponic growth of plants (Goddek, Joyce, Kotzen, et al., 2019). Aquaponics systems contain three main biological components; fish, plants, and beneficial bacteria (Figure 3). The fish eats feed and produces nutrient-rich feces and ammonia (NH_3) over the gills, which are released into the production water (Rakocy et al., 2006). The water flows to a plant section (hydroponics), where the plants absorb nutrients enabling plant growth. NH_3 is not available for the plants and beneficial bacteria are necessary to obtain a nitrification process (Anderson et al., 2019).

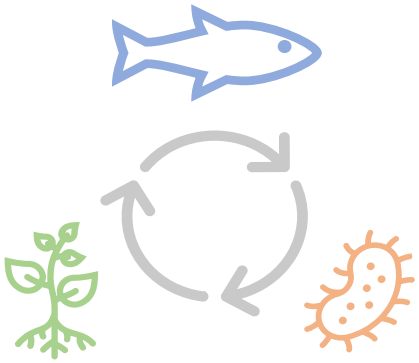

Figure 3 – Main elements of aquaponics; fish, nitrifying bacteria and plants.

2.3.1 Nitrogen Compounds and Nitrification

Nitrification is a two-step biological oxidation process of NH_3 to nitrite (NO_2^-) by NH_3-oxidizing microorganisms (AOM) such as *Nitrosomonas* and *Comammox* bacteria (Eq.1), then from NO_2^- to Nitrate (NO_3^-) by NO_2^--oxidizing bacteria (NOB) such as *Nitrobacter*, *Nitrospira*, and *Comammox* bacteria (Eq.2) (Ward, 2008). Newer research also shows that *Comammox* can oxidize NH_3 to NO_3^- completely. (Shi et al., 2020).

Nitrification process equations:

$$2NH_3 + 3O_2 \rightarrow 2NO_2 + 2H^+ + 2H_2O \qquad \text{Eq. 1}$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \qquad \text{Eq. 2}$$

The nitrifying bacteria can be found on roots, in the substrate or in biofilters. For larger systems biological filters (biofilters) are added before the hydroponics to secure sufficient nitrification (Espinal & Matulić, 2019). Without the AOM and NOB bacteria, the system would fail as the

plant cannot remove the nutrients, and NH_3 and NO_3^- are harmful to the fish even at low levels. Nitrate (NO_3^-), is more tolerated by many aquaculture species (Nelson, 2008).

The main part of N the fish excretes in RAS is excreted as TAN (Total Ammonium Nitrogen). The combination of ammonia–nitrogen (NH_3-N) and ammonium–nitrogen (NH_4^+– N) is called ammoniacal–nitrogen or TAN (Hagopian & Riley, 1998). In addition, other nitrogenous waste products are broken down to TAN by bacteria naturally occurring in the water. TAN will occur in two different forms, unionized NH_3 and ionized NH_4^+. The pH value in the water determines the ratio between NH_3 and NH_4^+ and is of high importance in live fish systems (Fjellheim et al., 2016). High values of NH_3 are toxic to fish as NH_3 is uncharged and lipid-soluble and can cross biological membranes. Thus, the pH determines whether a given TAN value is toxic or not (Downing & Merkens, 1955; Körner et al., 2001). Biofilters are therefore essential in RAS to convert NH_3 to NO_3^-, through nitrification (Fjellheim et al., 2016). Anderson et al. (2019) states that aquaponics systems usually have a pH around 7, meaning that 0,5% of the TAN will be NH_3, while the rest is NH_4^+.

2.3.2 System Structure – Coupled and Decoupled Systems

There are four main components in aquaponics systems; fish production, solid removal, biofilter and hydroponic plant section (Yogev et al., 2016). There are different ways to integrate hydroponics in aquaculture, either as coupled or decoupled systems. A coupled system is an aquaponics system with a full, closed loop. A continuous stream of water circulates between the fish and plants. The water goes from the fish tank, through filtration, over to the hydroponic section, and back to the fish as seen in Figure 4. Pipes are used to connect the different elements and create a closed water cycle. A pump sump is often added, where the water collects before being pumped back to the fish. Water then flows through the system due to gravity, having the sump as the lowest point (Palm et al., 2019). A closed aquaponics system intends to purify the water used in aquaculture production, by adding a plant section that filters the water. Adding plant species can also add economic benefits, by utilizing the waste product in a circular production (Palm et al., 2019).

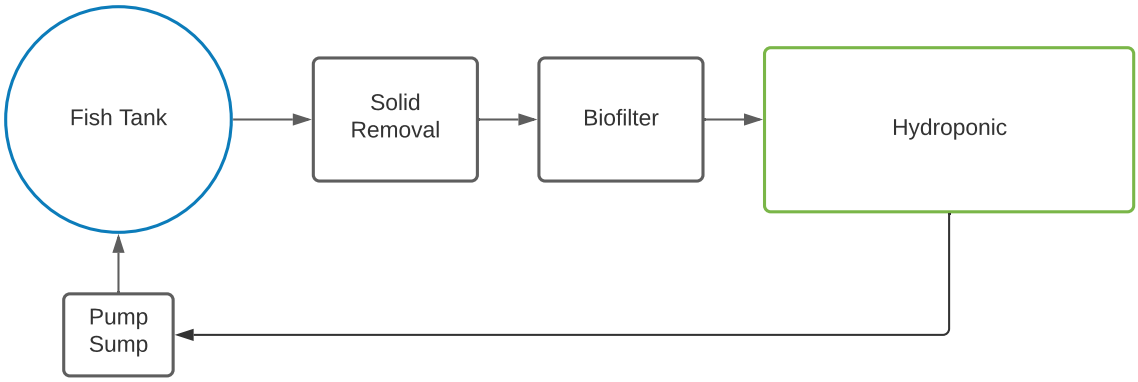

Figure 4 – A coupled system adopted from "Coupled Aquaponics Systems" by Palm et al. (2019). Water flows from the fish tank to the sump by gravity.

Plant and fish require different nutrient and environmental conditions, leading to a compromise in water quality and conditions. This can limit the efficiency of growth for both fish and plants, as neither has optimal conditions (Goddek, Joyce, Wuertz, et al., 2019). Such conditions can be water temperature, pH, salinity, dissolved oxygen (DO), electrical conductivity (EC) and NH_3. Lack of iron and potassium in the water has often been observed, but can be added to coupled systems without affecting the fish (Eck et al., 2019; Schmautz et al., 2016).

In decoupled systems, extra nutrients can be added to improve the nutrient solution to better match the plants' nutrient requirements (Goddek et al., 2015). The water does not flow directly between the fish and plants (Figure 5). Several steps can be included to optimize the water quality for both fish and plants, separately. Necessary nutrients can be added without affecting the fish through a water control system (sump and gas control before hydroponics). Here pH, temperature and nutrients are adjusted to suit the plant species. It provides increased stability in water quality, as well as opportunities to grow a greater variety of plants (Yep & Zheng, 2019). It also allows for higher recycling of nutrients from sludge through mineralization (Goddek, Joyce, Wuertz, et al., 2019).

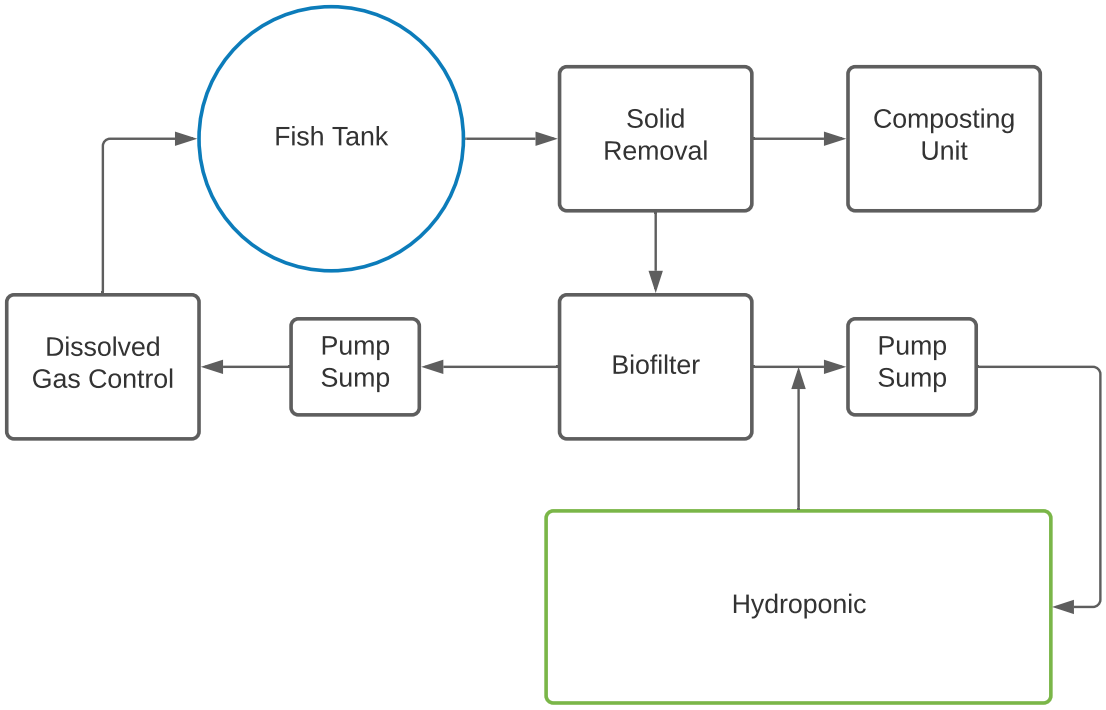

Figure 5 - Decoupled system based on "Aquaponics Guidelines" by Þórarinsdóttir et al. (2015)

2.3.3 Mineralization

Bioreactors can be included in decoupled systems to mineralize sludge and components such as phosphorus, magnesium, iron, manganese and sulfur. Minerals in the sludge are mostly insoluble components, which are not available to plants (Goddek et al., 2016). Mineralization can reduce waste as well as the need for commercial fertilizers (Delaide et al., 2019). Anaerobic and aerobic sludge digesters break down the sludge with the help of microorganisms (Delaide et al., 2018; Monsees et al., 2017). Mineralization can be done through an up-flow anaerobic sludge blanket (UASB) reactor where the sludge is broken down to bioavailable nutrients (Goddek et al., 2018). Microorganisms degrade the organic material and release ions at the right pH of plant uptake before the water enters the hydroponic system (Delaide et al., 2017; Seawright et al., 1998). The nutritious water will go from the bioreactor to the plants, without being connected to the fish production (Goddek, 2017; Goddek et al., 2018). A UASB has been shown to reduce up to 90% of total suspended solids (TSS) in aquaculture sludge treatment (Mirzoyan & Gross, 2013). The inclusion of an expanded granular sludge bed (EGSB) reactor can further treat the water coming from the UASB, to obtain a close to complete total TSS removal. The EGSB can remove remaining organic materials such as volatile fatty acids

(Ratanatamskul & Siritiewsri, 2015). Bioreactors can thus reduce waste production and secure optimal reuse of nutrients (Goddek et al., 2016).

2.4 Plants

Plants are a central part of an aquaponics system, as they absorb nutrients and filter the production water for the fish. For plants to grow, the right nutrients must be available, in the right form. Plant nutrients and aspects related to this will be further explained in this section.

2.4.1 Plant Nutrition

There are 17 essential nutrients needed for plant growth, divided into macronutrients and micronutrients. The three most important macronutrients are nitrogen (N), phosphorus (P), and potassium (K), which the plants need large doses of to grow. Calcium (Ca), magnesium (Mg) and sulfur (S) are other macronutrients needed in smaller doses (Department of Primary Industries, 1992; Maathuis, 2009). In addition, micronutrients such as iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), boron (B) and molybdenum (Mo) are needed in small doses to complete processes in the plants (Department of Primary Industries, 1992). Carbon (C), hydrogen (H) and oxygen (O) are nutrients the plants absorb from air and water (Adnane et al., 2018). An overview of the nutrients, the function it has in the plant, and the form of plant uptake can be found in Appendix 1.

Nitrogen (N) is an element that is essential to life and plant growth, as it is a part of proteins, nucleic acids and other vital organic compounds (Liu et al., 2014; Ohyama, 2010). The element occurs in many forms, in which it continuously cycles between with the help of bacteria. The nitrogen cycle has five main stages, as listed by Dodds and Whiles (2020):

- Nitrogen fixation from nitrogen gas (N_2) to NH_3/NH_4^+ or NO_3^-
- Nitrification from NH_3 to NO_3^- (Further explained in section 2.3.1)
- Assimilation through incorporation of NO_3^- or NH_3 into biological material
- Ammonification through the transformation of organic nitrogen compounds to NH_3
- Denitrification through transformation of NO_3^- to N_2, which is then released into the atmosphere

Phosphorus (P) is a fundamental element to all living organisms, as it is essential in building DNA and cell membranes. The global P is being used at a higher pace than it can be replenished,

meaning it is in danger of depletion. Agriculture utilize 90% of P through fertilizers (Reijnders, 2014). Actions to reduce the utilization of fossil P are therefore necessary. Reijnders (2014) states that an increased efficiency of P-use in agriculture, along with a higher degree of recycling of the element, is needed to reduce the extraction of fossil P. Traditionally, secondary P resources (human and animal excrete, harvest residues, organic wastes, ashes, and crushed bones) has been used for plant production (Ashley et al., 2011; Kirchmann et al., 2005).

Liebig's Law of the minimum states that the nutrient in the lowest supply related to the plant's requirements is the nutrient limiting the plan's growth (Ågren et al., 2012). Another theory is the multiple limitation hypothesis (MLH) which states that plants modify their growth patterns so that they will be limited by several nutrients at the same time (Ågren et al., 2012). Soil plants are often affected by the limited availability of N and P. In aquaponics systems without the addition of nutrients, P is often the limiting nutrient (Graber & Junge, 2009; Seawright et al., 1998). Eck et al. (2019) state that plants must be provided with sufficient levels of all important nutrients, to ensure plant yields. An optimal hydroponic plant solution is given by Hoagland (Table 1).

Table 1 - Optimal hydroponic nutrient solution; Hoagland No.2 adopted from "Complete guide for growing plants hydroponically" by Jones Jr (2014)

Nutrients	**Hoagland nutrient solution (mg/l)**
NO_3^-	220
NH_4^+	12,6
P	24
K	230
Ca	179
Mg	49
S	113
B	0,45
Cu	0,02
Mn	0,05
Mo	0,0106
Zn	0,48

EC is "*an index of salt concentration and an indicator of electrolyte concentration of the solution*" (Ding et al., 2018). The EC is related to the number of ions available for the plants in the nutrient solution and is often used as a measurement to control nutrient concentration. The optimal EC differs between species and environmental conditions. (Le Bot et al., 1998;

Sonneveld & Voogt, 2009b). High EC can affect the plants by hindering nutrient absorption due to increased osmotic pressure in the nutrient solution, while low EC can affect the plant health and yield (Ding et al., 2018). In a hydroponic system the optimal EC of nutrient solutions is 1,5 to 3,5 mS/cm, while for aquaponics optimal growth is seen with 0,3 to 0,6 mS/cm (Hess-Erga et al., 2013).

2.4.2 Lettuce (*Lactuca sativa*)

Lettuce (*Lactuca sativa*) are plants in the family *Asteraceae*, which are considered the most important leafy vegetables (Křístková et al., 2008). The plants are easily grown in cool weather with a pH of 6,0 to 8,0, to secure the uptake of macronutrients (Delaide et al., 2016). Lettuce grown hydroponically has a faster growth rate compared to traditionally grown lettuce and can be harvested after 35 to 40 days. In a nutrient film technique (NFT) system, more than 8 crops can be harvested a year (Touliatos et al., 2016). Maboko and Du Plooy (2009) report increased yield in lettuce grown in a recirculating hydroponic system with a density of 50 plants per m^2. Morgan (1999) recommends an EC of 2-2,5 mS/cm for lettuce during the production phase.

Leafy greens contain around 4% dry matter (Anderson et al., 2017). Petek et al. (2020) found through their literature review that N content range between 1,13 to 5,02% of dry matter in lettuce (leaves), while Maynard et al. (1976) states that around 4% of dry matter in lettuce is NO_3^-. For P the numbers were 21 to 68% of fresh weight, while Estrada et al. (2016) states that it accounts for up to 0,2% of dry matter in lettuce.

2.5 RAS-technology

A simplified and generalized flow chart of RAS is presented in Figure 6, to explain how the technology functions. The flow chart is based on processes provided by RAS suppliers AkvaGroup and AquaMaof (AquaCon, 2022; AquaMaof, 2019). The inputs in the fish tanks are clean water, fish, and feed. Waste products accumulate in the fish tanks as the fish consume feed and produce excrete. Fjellheim et al. (2016) state that 50% of particles are removed in the tank thorugh the outlet, which reduces the accumulation of dissolved particles. Recommended water quality parameters for RAS with salmonids are listed in Appendix 2 based on Ebeling and Timmons (2010).

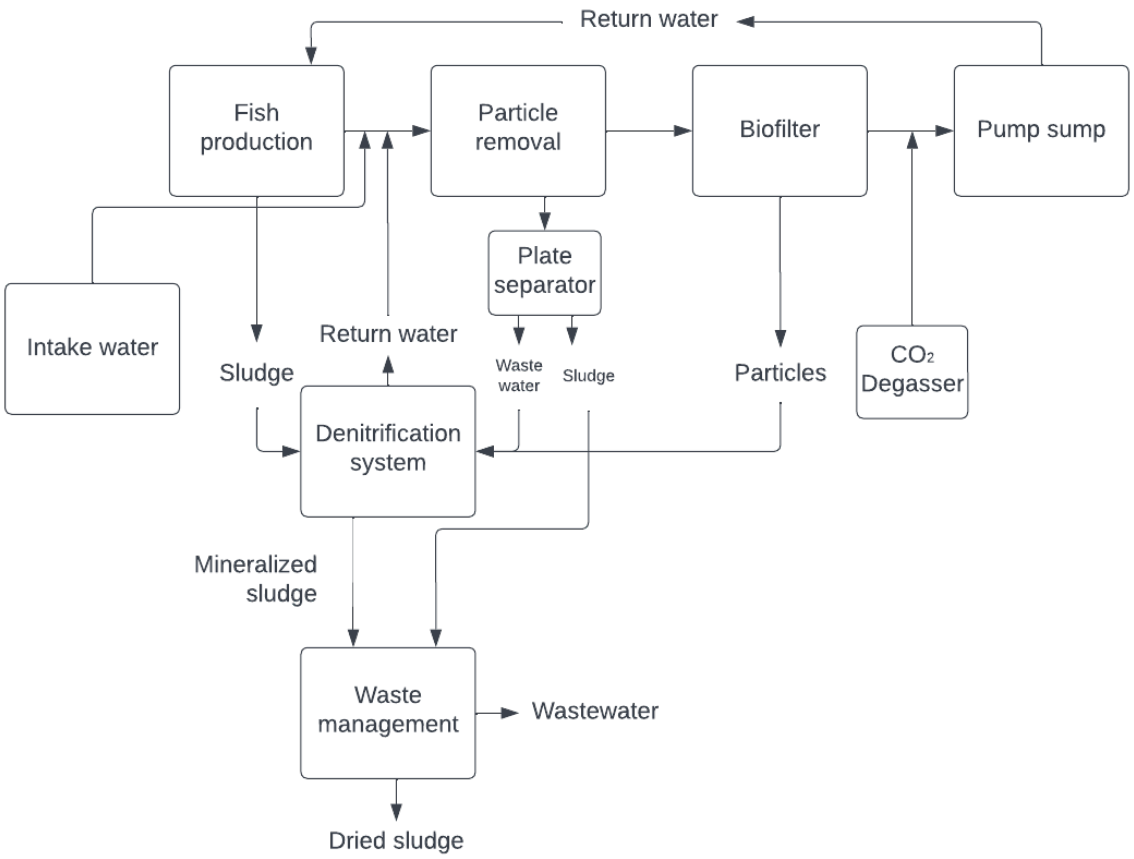

Figure 6 – Simplified and generalized flow chart of recirculating aquaculture system (RAS) production, adopted and modified from RAS processes by AquaMaof and AkvaGroup. Plate separator is included to show its place in RAS, even though AquaMaof does not use this technique (AquaCon, 2022; AquaMaof, 2019).

The RAS includes all steps to clean the water before it can return to the fish tank. A variety of systems exist, some utilizing more advanced processes to reuse high amounts of water, called zero-water exchange systems. Figure 7 presents the correlation between degree of recycling and complexity of processes needed, which will be further mentioned in section 2.5.4. AquaMaof (2019) states a removal of 5-10% of the total water volume to the denitrification system (DNS) daily, together with the particles accumulated in the outlet and settlers (AquaMaof, 2019). The goal is to dilute NO_3^-, remove NH_3 and secure a safe environment for the fish. The main components included in RAS, relevant for

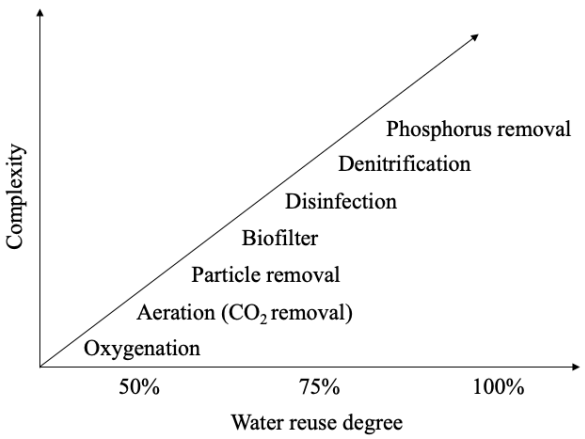

Figure 7 - The correlation between degree of recycling and the complexity of processes needed to achieve this, in recirculating aquaculture systems. Based on Fjellheim et al. (2016).

aquaponics, are described in this section; particle removal, biofilter, pump sump, and denitrification and de-phosphorus system.

2.5.1 Particle Removal

Solid removal is seen as one of the most critical processes in aquaculture systems. Large portions of solid waste are removed through settling in the tanks, but finer particles must be removed through further mechanic filtration, or in some cases settling chambers (Piedrahita, 2003). Settling chambers are tanks without turbulence, where the particles can sink to the bottom and then be removed. The most used mechanic filters are screen filters and expandable granular media filters (Ebeling & Timmons, 2012). Martins et al. (2010) state that RAS removes approximately 85-98% of suspended solids and organic matter and 65-96% of P through particle removal.

2.5.2 Biofilter

A biofilter is included in RAS to support the nitrification process of converting NH_3 to NO_3^- (Section 2.3.1) (Palm et al., 2019). The biofilters are structured using inert materials providing a large surface area for the biofilm of nitrifying bacteria to oxidize NH_3-N (Anderson et al., 2019) (Fjellheim et al., 2016). A biofilm is a complex of bacteria embedded in a matrix of extracellular material consisting of different types of biopolymers. It forms a three-dimensional architecture that sticks to surfaces and allows for a more stable lifestyle, with cell-to-cell communication, nutrient exchange and it makes bacteria more resistant to stress from the environment (Flemming & Wingender, 2010). To achieve a good conversion of NH_4^+ to NO_3^-, the nitrifying bacteria must have an environment with optimal conditions.

2.5.3 Pump Sump

Pump sumps are tanks where water is collected before it is pumped back to the fish tanks.

2.5.4 Denitrification, Sludge Decomposition, and Waste Management

In some RAS facilities NO_3^- is removed from the water by bacteria through anaerobic processes in biofilters. Some autotrophic (*Thiomicrospira, Thiothrix, Rhodobacter, Hydrogenophaga*) and heterotrophic (*Pseudomonas, Paracoccus, Comamonas*) bacteria can use NO_3^- to oxidize their energy source under anaerobic conditions, giving an end-product of N_2 which is released into the environment (Fjellheim et al., 2016; Rurangwa & Verdegem, 2015). The anaerobic

biofilter is used on a side stream of water, and biological matter is added as an energy source. Close control is important so toxic compounds such as hydrogen sulfide (H_2S) do not develop. A denitrification system can lower the need for water removal/new water intake by 90%. (Fjellheim et al., 2016). A sequencing Batch Reactor (SBR) can be used to perform denitrification, with sludge as a carbon source. By adding the sludge, a process of decomposition and mineralization will occur (AquaMaof, 2019). A fluidized Sand-Bed filter (FSBR) can be used to further reduce NO_3^-, NH_3, and other biodegradable organic matter, through biofilm formation on particles (AquaMaof, 2019).

In AkvaGroups zero-water exchange system the products from the drum filter (mechanic filter) will continue to a plate separator. Here particles settle and are pumped to waste management, while water can continue to chemical cleansing (Wæhre, 2019). The water found high in the water column of the plate separator has fewer particles, but high amounts of nutrients such as NO_3^-. The water continues to a biofilter for denitrification, then to a system for de-phosphorus. Here iron chloride is added to precipitate the P, which can then be removed from the system, and water can return to the loop (AquaCon, 2022).

Waste products are removed during several steps in the RAS and goes through processes to decompose and mineralize the waste, yet some compound cannot fully be degraded and must be removed from the system (AquaMaof, 2019). The sludge is mechanically filtered in a belt filter to increase the amount of dry matter in the sludge (to appx. 10%), and centrifuges can further dry the waste (to appx. 20%) (AquaMaof, 2019).

3 Material and Methods

This thesis has used two different approaches to gain more understanding of the research question. A literature review was made to find the potential for plant growth in RAS through published research papers, and an experimental approach was included by setting up a plant experiment to see the actual effect. The methods will be further explained in this chapter, and Figure 8 provides an overview of the methods used.

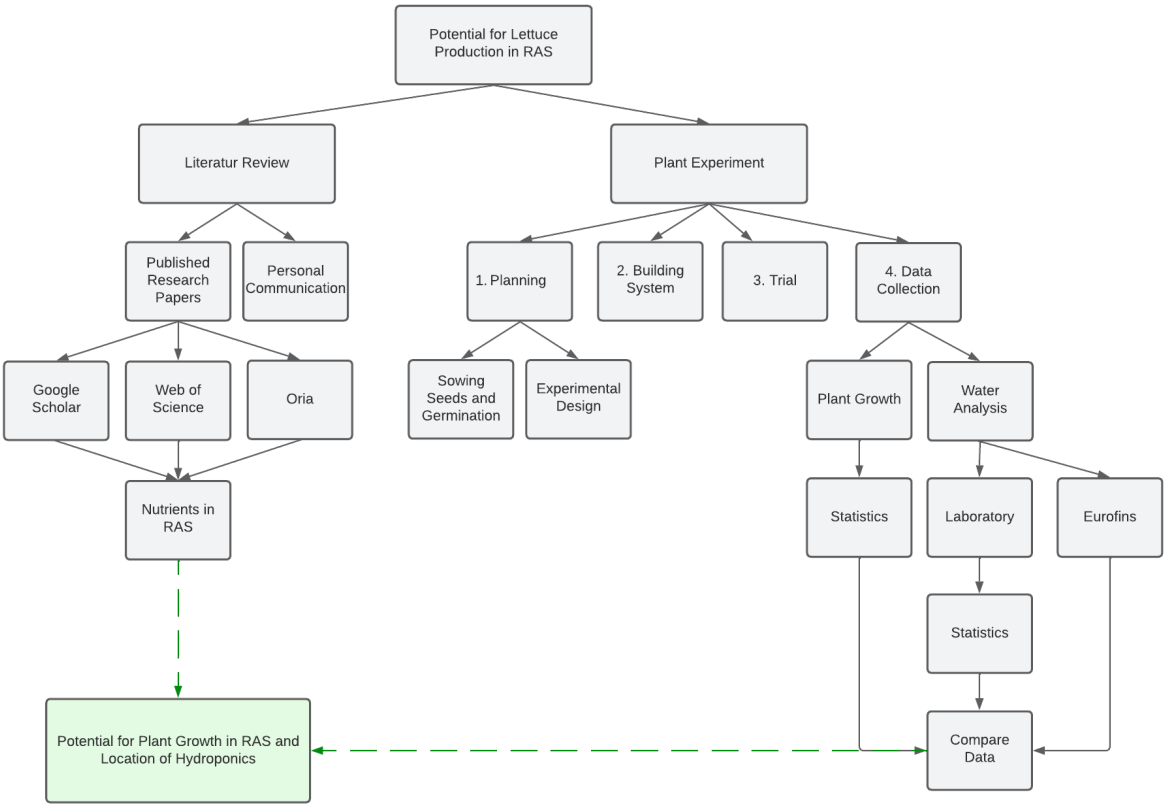

Figure 8 - Overview of the experimental setup and methods used to conduct the present Master of Science.

3.1 Literature Review

A systematic literature search was performed, firstly in the database 'Web of Science' (Clavariate Analytics, United States), then in Google Scholar and Oria, to explore the potential for plant production in RAS.

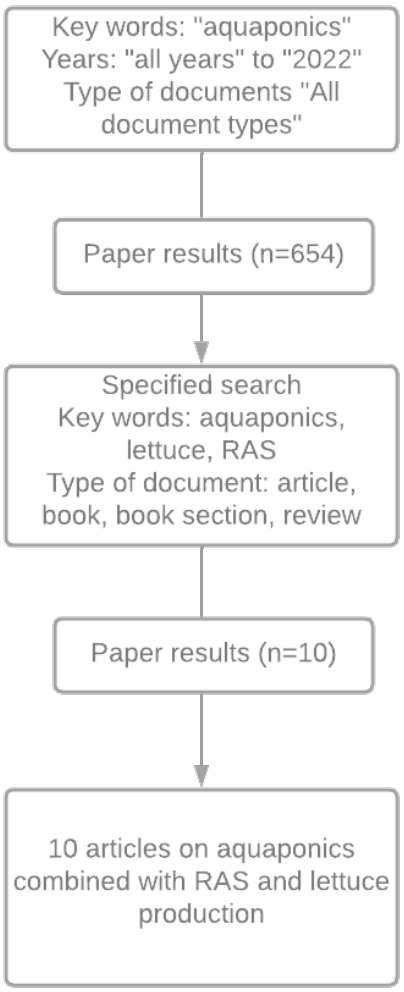

Figure 9 - Available articles on the relevant topic from searches in Web of Science

The initial search on the combined keywords "aquaponics", "RAS" and "lettuce" / "*Lactuca sativa*", gave few results (n=10). Figure 9 shows how the initial search was performed. The literature available was related to warm water species, and not directly relevant to salmonids. Aquaponics in combination with RAS and salmonids is a newer phenomenon, currently getting increased attention. All relevant articles are published from 2017 to now (2022).

The search was expanded, and more specified on the different topics. Several combinations of the keywords ("*Lactuca sativa*", salmon, hydroponics, aquaponics, nutrient uptake, RAS (P and N in RAS) and wastewater) were used to find relevant literature to gather an understanding of problem statement. License applications and personal communication with Norwegian RAS producers were used to find further data on nutrient concentrations in RAS. Expert opinions have been gathered from qualified people within the fields of RAS and aquaponics (Appendix 3).

The results from the literature review are located in the first section of the results chapter, as a large amount of data and information is presented. This section is meant to provide a better understanding of the potential of including a plant section in RAS. A table was formed to present data on nutrients in different processes of RAS.

3.2 Plant Experiment

A plant experiment was structured to research the filtering effect lettuce could have in different types of water from RAS. The experiment took place at Val VGS (64°47′09′′N 11°25′24′′E) at Kolvereid, Norway in February 2022.

3.2.1 Biological Material and Preparation

The biological material used in the experiment was Lollo Bionda lettuce (*Lactuca sativa* var. crispa) and water from freshwater RAS production of salmon smolt. Lollo Bionda is a leafy lettuce type, with a light green color, frilly leaves, and an average harvest mass of 300g. Leaf lettuce does not form solid heads, but rather a group of leaves (Koudela & Petříková, 2008). The plant can be grown throughout the year in different systems and is a popular hydroponic species. The lettuce quality is affected by light quality and fertilizer type (Draghici et al., 2016).

148 Lollo Bionda (*Lactuca sativa* var. crispa) seeds were sown on the 7th of January 2022, in stone wool presoaked in water with Root!t first feed solution (6 ml/l) (Mikrogartneriet, 2022). Two seeds were sown in each of the 74 stone wool cubes (Figure 10). 24 seeds were sown in a 12-pot sowing tray with germination soil, to be used as control plants. Seeds were covered in plastic foil until germination (after 3 days). Artificial LED lights (Nelson Garden, 85 cm, 23 W, 130 µmol/s/m²) (Nelson Garden, 2022) were added, as 'Lollo Bionda' can grow 30% better with LED than neon lights (Panter et al., 2015). The stone wool was kept humid with the Root!t solution, after germination. Soaking stone wool and irrigating sprouts with diluted hydroponic solution will prepare the roots and plants will adapt more easily to the hydroponic solution (Morgan, 1999). If both seeds germinated, one of the sprouts was removed. Soil plants were irrigated with room-tempered tap water and kept humid. Temperatures were steady at 21,5°C. When plants had approximately 4 leaves, they were transferred to deep water systems with water from RAS (after 26 days).

Figure 10 - Pictures showing the sowing process of lettuce seeds in stone wool.

RAS water contains valuable nutrients as mentioned in section 2.1.3. The water used in the experiment was collected from Midt-Norsk Havbruk AS (MNH) smolt facility Osan, which has a zero-water exchange system from AkvaGroup. Water was extracted from after the biofilter (growth media 1 - GM1) and the top of the plate separator (Growth media 2 - GM2). The extraction was performed using a submersible pump to fill two disinfected 200l barrels, with 150l of each water type. Table 2 lists some of the physiochemical parameters in the two water types used in the experiment. Content of the RAS water is found in Table 5 in the result section 4.2.3, where input, output and decrease of nutrients are presented together to give a better overview.

Table 2 - Physiochemical parameters in the water from Osan smolt facility.

Parameters	**Growth media 1 (After biofilter)**	**Growth media 2 (Plate separator)**
Temperature (°C)	21	21
Dissolved oxygen (mg/l)	11,4	8,9
pH	7,3	7,2
Conductivity (EC) (mS/cm)	4,38	4,19

3.2.2 Experimental Design

A simple deep-water culture system (DWS) was made according to Figure 11 and Figure 12. Six plastic containers (40l) were used. Floating rafts were made by drilling 9 holes in each of the 6 expanded polystyrene sheets (EPS) (20 mm thick), using a hole saw (Ø50 mm). Net pots were placed in each hole. Two air pumps were used to provide aeration in the containers (BOYU S-4000B). Air hoses (1,25m) were connected to 6 air stones which were placed in each container. The setup consisted of two different growth media, with three replicates each. Growth media 1 (GM1 - replicates 1-A,1-B,1-C) was filled with water from after the biofilter (35l), and growth media 2 (GM2 - replicates 2-A,2-B,2-C) with water from the plate separator (35l). When the water reached room temperature, plants of average size and leaf development were transferred to the system and randomly placed in the 6 replicates. Giving a total of 54 plants in the hydroponic system.

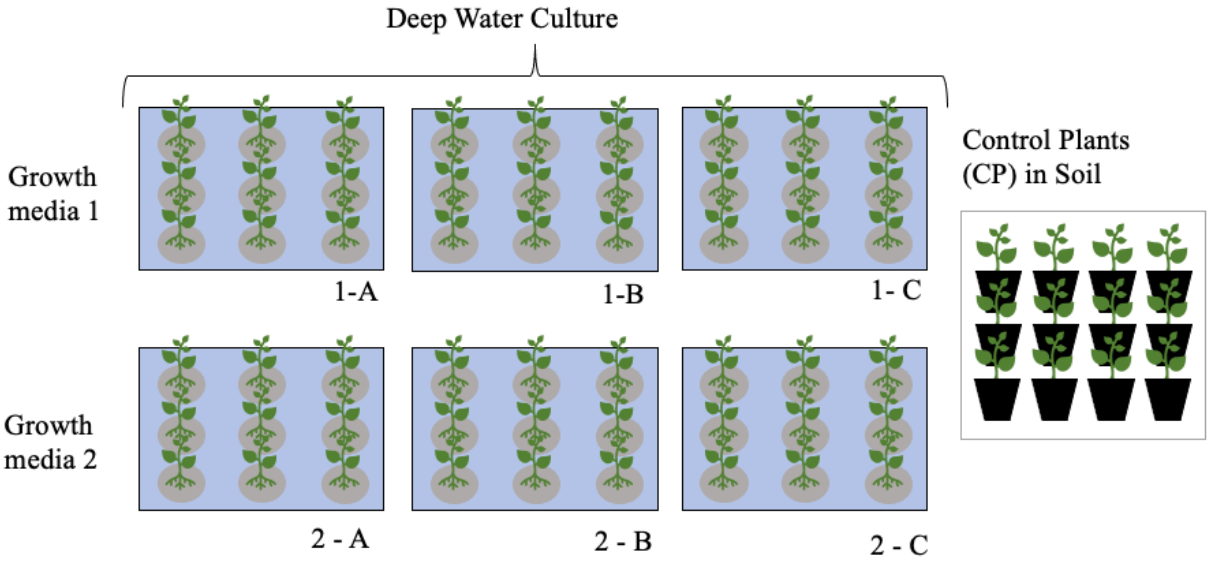

Figure 11 – The planned experimental setup of a deep-water system for Lactuca Sativa, showing hydroponic plants and control plants in soil.

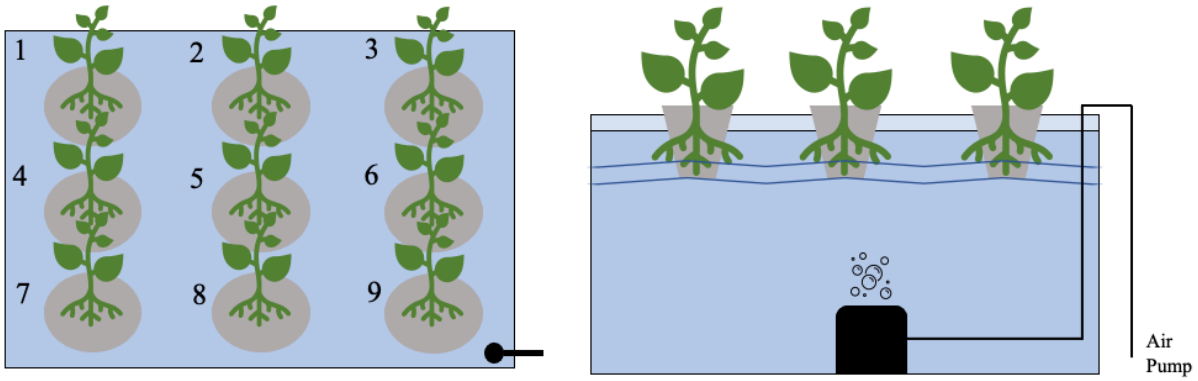

Figure 12 - Overview of the experimental system for Lactuca Sativa in a deep-water system, seen from above and the side.

Soil plants (CP) were repotted into pots of Ø120 mm, with premium plant-soil which was kept humid. Soil plants had the same light and temperature conditions as the hydroponic plants and were used as a control group.

3.2.3 Experiment and Data Collection – Plants

Room temperature was 22,5±2,5°C during the experiment. Artificial lights were added 15 hours a day, regulated by a timer. All plants were measured for height and weight directly before plants were added to the system. Aerial height and root length were measured using a folder ruler. For aerial height, the length was measured from the top of the stone wool to the end of the longest leaf. Root length was collected by measuring from the top of the stone wool to the end of the longest root. Accurate to the nearest millimeter. Weight was measured when the stone wool was completely soaked (Senz SESC30WH). The numbers of leaves were counted and registered.

The experiment ran for 7 days. Measures of dissolved oxygen, saturation, and water temperature (Oxyguard Handy Polaris), pH (HORIBA LAQUAtwin-pH-11), EC (HORIBA LAQUAtwin-EC-11), and room temperature were registered according to Table 3. Pictures were taken of each hydroponic system of the aerial part of the plants, as well as root systems. Water samples were collected for further analysis at the Norwegian University of Science and Technology (NTNU) laboratory (section 3.2.4). Personnel at Val FoU and Val VGS helped with the daily care of the plants and registration of data from day 2 until day 6, with the exception of days 3 and 4.

Table 3 – Plan for data collection of physiochemical water parameters during the experiment.

	Air temperature	**Water temperature**	**pH**	**Dissolved oxygen**	**Electrical conductivity**	**Pictures**	**Water samples**
Day 0	x	x	x	x	x	x	x
Day 1	x	x		x			
Day 2	x	x	x	x	x	x	
Day 3							
Day 4							
Day 5	x	x	x	x	x	x	x
Day 6	x	x		x			
Day 7	x	x	x	x	x	x	x

To show the system and plants, pictures are provided. Figure 13 show the system itself, measures of root length and of water parameters with a Oxyguard.

Figure 13 – The four pictures on the left are presented to show the actual setup of system, measuring of plants and water parameters, pictures on the right show replicate 1-C and three control plants.

Figure 14 show differences in the water reservoir for plants in 1-C and 2-B.

Figure 14 – The pictures show differences in water at day 7 of the experiment, for replicate 1-C and 2-B.

After removing plants from the system, all plants were measured for height (aerial and roots) and fresh weight (aerial parts, roots, and stone wool). Measures accurate to 0,0001g (Sartorius BP analytical balances, Model BP221S) (Sigmaaldrich, 2022). The aerial part was removed from the roots and stone wool by cutting the plant directly above the stone wool. Five pieces of

soaked stone wool were weighted to get the average stone wool weight. This weight was subtracted from the total weight to see plant weight without stone wool. Only aerial height and weight were registered for soil plants. The numbers of leaves were registered. Plants were placed in plastic bags and transferred to the laboratory to find dry matter (DM) and ash content (AC).

The DM content was found using the official method given by AOAC (2005). Porcelain crucibles were weighed before and after one lettuce plant was added. Measures accurate to 0,0001g. The plants were dried at 105°C for 24 hours, to get the dry weight. The dry samples were weighted before a muffle furnace (Nabertherm, Muffle Furnace up to 1400°C) (Nabertherm, 2022) was used to find the AC at 550°C degrees for 20 hours. The crucibles were weighed to determine ash content. Figure 15 show the plants at different stages of the drying and burning process.

Figure 15 - The pictures show the process from fresh weight (left) to dry matter (middle) and to ash content (right).

The findings from the data collection of plants are represented as average and standard deviation ($\bar{x}$ and SD) of the replicates and of the different growth media. DM and AC is presented as $\bar{x}$ and SD in percent of fresh weight lettuce leaves based on the modified calculations seen in Eq. 3, Eq. 4 and Eq. 5 (AOAC, 2005) (Horwitz, 2010). Two missing data points occurred due to mistakes made during drying of samples.

$$\% \, Dry \, matter = 100 - (\frac{(WWS - WSAD)}{WWS} * 100\%) \quad \text{Eq. 3}$$

$$\% \, Ash \, content \, of \, FW = 100 - (\frac{(WWS - WSAB)}{WWS} * 100\%) \quad \text{Eq. 4}$$

$$\% \, Ash \, content \, of \, DW = 100 - (\frac{(WSAD - WSAB)}{WSAD} * 100\%) \quad \text{Eq. 5}$$

WWS – Wet weight of sample
WSAD - Weight of sample after drying
WSAB – Weight of sample after burning

3.2.4 Data Collection - Water Samples

Water samples (50 ml) were collected in 50 ml plastic test tubes from each water container on day 0, day 5 and day 7 of the experiment. Water samples were frozen directly at -18°C. The frozen water samples (14 x 50 ml) were thawed in room tempered water and analyzed at the Food Science laboratory using HACH HQ40d multi (Colorado, USA). The calibration and measuring procedure given by HACH was followed for each probe, as seen in Appendix 5. Deionized water and lint-free cloth were used to rinse the probe before and after every use. Probe stands were used to keep the probes still during the read.

3.3 Water Samples Measured at Eurofins

Water samples were collected by personnel at a Norwegian RAS smolt facility, at two different sample points; after mechanic filtration and after biofilter. Samples were filled in 2l plastic containers from Eurofins. The water was analyzed to see the change in water after passing through the biofilter and to see the effect of nitrification. Parameters during production at the day of water outtake were; 31860 kg biomass, 450 kg feed, pH 6,6 (at tank outlet), 14,2 mg/l NO_3-N (after biofilter), intake water of 630,72 m^3/day and RAS-supplier Kruger Kaldnes. The smolt facility wish to remain anonymous (Anonymous, personal communication, February 2nd, 2022).

As mentioned in section 3.2.1, water samples were collected from MNH Osan smolt facility, for the water used in the experiments. The sample points were; water after biofilter (2l) and water from the plate separator (2l). Parameters during production at the day of water outtake

were; 214 000 kg biomass, 1379 kg feed, pH 7 (at tank outlet), 63 mg/l NO_3-N (after biofilter) and RAS-supplier AkvaGroup (Øren, S. O., Personal communication, January 21, 2022).

Two merged water samples of the outtake water from GM1 (1-A,1-B, and 1-C) and GM2 (2-A and 2-B) were collected. In GM1 6,7 dl was collected per replicate and in GM2 10 dl were collected per replicate. Samples were filled in 2l plastic bottles from Eurofins. All the extracted water samples were placed in the cooler bag on the day of extraction and sent by over-night mail to Eurofins Moss. The water analysis performed are listed in Appendix 6. All analyses were performed for water samples from Osan and the anonymous smolt facility. Water out of the experiment was not analyzed for Fe, Cu, Zn, pH, and SS.

3.4 Calculations on nutrient uptake

Plants uptake of NO_3^- and P was calculated based on the change in dry biomass. First the change (Δ) in biomass was found for each individual system. Then the average dry weight of the parallel was used to calculate the total dry weight biomass production for the 9 plants in the system. Next the amount of NO_3^- in the produced biomass was calculated as 4% of the DW (Maynard et al., 1976), followed by P accounting for 0,2% of DM (Estrada et al., 2016). NO_3-N content was found based on the total mass of N in NO_3, by multiplying NO_3 with 0,226 (HACH, 2022f). Similarly, PO_4-P is found by multiplying PO_4^{3-} by 0,3261 (HACH, 2022g). Calculations followed equation 6 and 7.

$$DM\ (g) = \frac{\bar{x}DM\ (\%)\ x\ D\ (g)}{100} \qquad \text{Eq. 6}$$

DM – Dry matter biomass (9 plants)
$\bar{x}DM$ – Average dry matter per growth media, in percent
D – Change in biomass (end weight – start weight)

$$NP\ (g) = \frac{NDM\ (\%)\ x\ DMPB\ (g)}{100} \qquad \text{Eq. 7}$$

NP - Nutrients produced
NDM - Nutrient concentration in dry matter
DMPB – Dry matter in produced biomass

The results were then compared to calculations on nutrient removal in the water. This was done by taking the nutrient content going in and out of the systems (in mg/l) and multiplying it by the volume of the system (35l) (Eq. 8). It is assumed that the nutrients have no other way out of the system, other than through plant uptake.

$$NR\ (mg) = DN(mg/l) x\ V(l) \quad \text{Eq. 8}$$

NR – Nutrient removal from the system (mg)
$DN - Change\ in\ nutrient\ content\ (mg/l)$
V – Volume in system (l)

3.5 Statistics

IBM SPSS statistics version 28 (IBM, New York, USA) was used to analyze statistics from the plant experiment. One-way analysis of variance (ANOVA) was used to determine the significant difference of the average between groups. All values are assumed to be within normal distribution. Tukey's honest significance difference (HSD) posthoc test are used to determine where the difference can be found at a 95% confidence interval (a=0,05). A p-value less than or equal to the alpha (a≤0,05), means the null hypothesis is rejected, and the result is statistically significant (Lucas, 2020). One-way ANOVA (OWA) was performed to find differences in plant growth, water parameters during the experiment, and water samples analyzed at the lab, for the three growth media. This is to check for any difference within the replicates or between the growth media. A general linear model (GLM) (univariate) was also used to analyze water parameters to check for significant differences based on the factors growth media and time during the experiment. For statistics related to weight, soil plants (CP) were excluded as the total weight could not be obtained without damaging the plants.

Pearson Correlation was performed to analyze the relationship between two factors. The correlation coefficient (r) can range from -1 to +1, -1 points to a negative correlation, and +1 to a positive correlation. 0 indicates no correlation. (UCLA, 2006) A positive correlation occurs if one variable increase as the second increases (or opposite). For a negative correlation, one variable decreases and the other increases (MedCalc, 2022).

4 Results

Results found through the literature review are firstly presented with a data collection on nutrients in RAS. Then results regarding the plant experiment are presented, with physiochemical water parameters, plant growth and nutrient uptake calculations. Finally, water samples from a RAS smolt facility (anonymized) are included to see the effect of a biofilter.

4.1 A Theoretical Approach to Explore the Nutrient Concentrations in Recirculating Aquaculture Systems

The amount of waste products collected from land-based facilities is expected to rise (Brod, 2021). Meriac (2019) estimated a total production of 8 000 tons of dry waste from feces in 2017, which will continue to increase rapidly. As mentioned, the sludge from RAS consists of uneaten feed and feces. Fish feed from Skretting Norway in 2019 contained 7,6 g/(kg feed) nitrogen in feces and 29,6 g/(kg feed) nitrogen dissolved in water, giving a total discharge of 37,1 g/(kg feed) nitrogen. The numbers for P were 5,5 g/(kg feed), 1,0 g/(kg feed) and 6,5 g/(kg feed), respectively (Lea, 2020). High amounts of solid waste is collected in RAS, but a significant amount of dissolved nutrients are still released to nature (Nibio, 2017). The wastewater is emitted when the level of nutrients and suspended particles are within the limits of the Pollution Control Act (Biogass Oslofjord, 2018). Nutrients such as N and P mainly follow the wastewater, while small parts remain in the dried waste product (Rambøll, 2019; Rosten, 2015). Nibio (2017) states that 27 000 tons of N and 9 000 tons of P end up in the ocean yearly due to land-based aquaculture in Norway. Jakobsen et al. (2021) reported a release of 50 000 tons of dissolved inorganic N to the environment due to Norwegian aquaculture in 2019. Inorganic N consists of N_2, NO_3^-, NO_2^- and NH_4^+ (Dodds & Whiles, 2020). Smolt facilities account for about 2% of the total production and stand for the emission of approximately 1 000 tons of dissolved inorganic N. This amount is more than what is needed for all greenhouse production in Norway (Jakobsen et al., 2021), giving an impression of the amount of N lost and the potential for nutrient recycling. Brod (2021) found that up to 70% of N available for plants, follow the wastewater.

Timmons et al. (2002) note that the sedimentable fractions contain the main part of the P emission (50-85%), while most of the N emission (75-80%) is dissolved NH_4^+ or NO_3^- (if nitrification occurs) (Hess-Erga et al., 2013). Osan smolt facility plan to remove 99% of all total

N and 24% of total P from the effluent water before the water can be released to the recipient (Wæhre, 2017). Data on nutrient concentration in water at different stages in RAS are listed in Table 4 and show the most important findings based on literature review and water analysis.

Table 4 - Nutrient contents in different parts of recirculating aquaculture system (RAS) production with Atlantic Salmon, based on literature review and water analyses.

Point during production (mg/l)	Total N	Nitrite (NO_2-N)	Nitrate (NO_3-N)	Nitrite + nitrate - N	TAN	Total P	Literature
Intake Water (RV)[1]		<1,0	0-150		<1,0	0,01-3,0	Ebeling and Timmons (2012)
Fish Production		0,01 ± 0,00	19±2		0,11±0,01	0,9±0,1	Davidson et al. (2016)
	2,58±2,55						Rosten (2015)
		0,03±0,02	17,2±11,9		0,12±0,02		Mota et al. (2019)
		0,023±0,006	22,56±6,20		0,21±0,04		Kolarevic et al. (2014)
After mechanic filter	31		0,33			32	Cabell et al. (2019)
	16		0,005			8	Cabell et al. (2019)
	2,80±3,42						Rosten (2015)
	15	0,17	14,2		0,573[3]	2	WS[4] Smolt facility
After biofilter (incl. pump sump and return water)		0,23±0,09	25,54±2,10		0,73±0,28		Dahle et al. (2022)
	37		9,9			11	Cabell et al. (2019)
	15	0,17	14,2		0,17[3]	1,8	WS[4] Smolt facility
	51			37[2]	0,31[3]	7,4	WS[4] Osan Smolt facility
Plate separator	53			34[2]	4,5[3]	10	WS[4] Osan Smolt facility
Wastewater	20					2,2	Hess-Erga et al. (2013)
	46					62,2	Hess-Erga et al. (2013)
			50		0,7		Kjos-Hanssen (2021)
	15					20	Kvidul (Steinke, D., pers.comm. 2022)
Sludge						0,85	Ytrestøyl et al. (2013)
						0,68	Ytrestøyl et al. (2013)
						1,5	Ytrestøyl et al. (2013)

[1]RV=Recommended values
[2]Combined measure of NO_2-N and NO_3-N
[3]The value for ammonium (NH_4^+) is listed as TAN, as TAN will only consist of 0,0037% ammonia (NH_3-N and Cl) per mg/l at pH 7. Both samples had a pH below 7.
[4]WS=Water samples taken during the project

The data collection shows varying levels of total N for the different systems, with the highest concentration being found in the plate separator (53 mg/l), for the water used in the experiment. From published literature, the highest amount of total N is found after biofilter (51 mg/l) and in wastewater (46 mg/l). A closer look at the N compounds shows that water after the biofilter has the highest concentration of NO_3^- (25,54±2,10 mg/l), followed by the water in fish production (22,56±6,20 mg/l). TAN, mainly as NH_4^+, is found in higher concentrations in the plate separator than anywhere else (4,5 mg/l). Water from after the biofilter contain from 0,17 mg/l to 0,73 mg/l TAN. For total P the highest concentration is found in wastewater (62,2 mg/l), then

after the mechanic filter (32 mg/l). The literature review shows lower levels for water after biofilter (1,8–11 mg/l).

4.2 Plant Experiment

Physiochemical parameters were tested during the experiment and water samples were analyzed both at the Food Science laboratory and by Eurofins. The results found are described in this chapter. Replicate 2-C was removed from the results due to errors during the experiment.

4.2.1 Physiochemical Parameters Observed During the Experiment

Physiochemical water parameters registered during the experiment are listed in Appendix 7. Changes in the parameters water temperature, EC, pH and DO for the 7 days can be seen in Figure 16. Measurements for CP were not included as soil plants were not influenced by the water parameters listed. No significant differences were seen between replicates within the growth media, for either of the water parameters (p>>0,05).

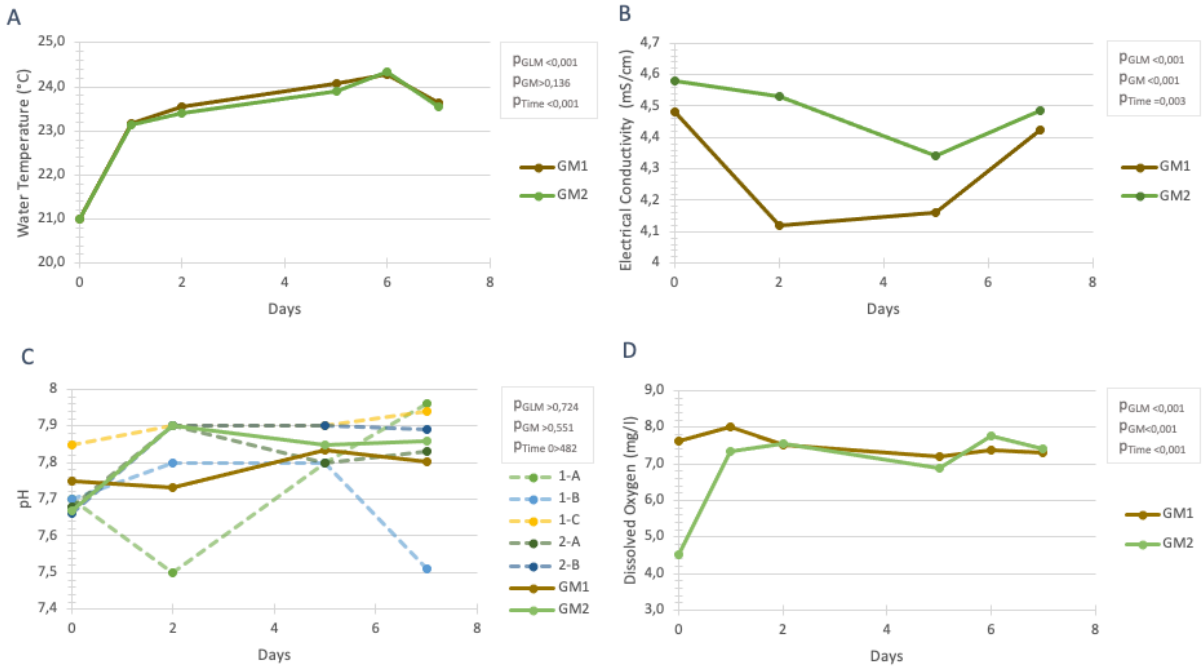

Figure 16 - Figures show the physiochemical water parameters measured over the 7 days the experiment ran, the two hydroponic growth media's (GM) average is presented GM1 (n=3) and GM2 (n=2). Figure A show water temperature development. Figure B show the change in electrical conductivity (EC). Figure C show the change in pH (including all replicates due to large differences within the growth media). Figure D show the change in dissolved oxygen (DO). Statistical analyses were conducted using a general linear model (GLM) selecting GM and time as fixed factors. Differences were found for water temperature, EC, and DO.

No significant differences were seen for most of the physiochemical water parameters between water from the biofilter (GM1) and water from the plate separator (GM2). For water temperature no significant difference was seen during the experiment ($p>0,900$), with average values of 23,38±1,11°C and 23,23±1,11°C for GM1 and GM2, respectively (Appendix 7). Figure 16-A show the trends in water temperature development during the experiment, showing significant differences as a function of time ($p<0,001$) but not for the factor growth media ($p>0,136$). For EC, a significant difference was seen between the growth media ($p=0,020$), where GM1 and GM2 showed average EC values of 4,30±0,19 mS/cm and GM2 4,49±0,1 mS/cm, respectively. Figure 16-B show the trends in EC development during the experiment, showing significant differences as a function of time ($p<0,001$) and for the factor growth media ($p=0,003$).

For pH no significant difference was seen between growth media ($p>0,524$). Average pH for GM1 and GM2 was 7,78±0,15 and 7,82±0,10, respectively, throughout the experiment. Figure 16-C show replicate 1-A having a drop in pH on day 2, and 1-B on day 7. Similarly, no significant difference was seen in DO during the experiment ($p>0,059$). Average DO for GM1 and GM2 was 7,5±0,35 mg/l and 6,91±0,90 mg/l, respectively. Figure 16-D show the trends in DO development during the experiment, showing significant differences as a function of time ($p<0,001$) and for the factor growth media ($p<0,001$). To see how different water parameters can affect each other, Pearson correlation was performed at 95% confidence interval (CI) (Appendix 8). No correlation was observed.

4.2.2 Water Samples Analyzed at NTNU's Food Science laboratory

Water samples were extracted on day 0, day 5 and day 7 of the experiment, and were analyzed for pH, temperature, NH_3-N, DO, and Cl. All data are listed in Appendix 9. To better visualize the result, graphs for pH, DO, Cl and NH_3-N are presented (Figure 17). Two water samples were taken at the start of the experiment, one for each growth media. Thus, no difference between replicates were expected within the growth media for start values. Temperature measurements of samples are most relevant when seen in combination with the other parameters, as they can be influenced by temperature. All measurements and p-values are listed in Appendix 9.

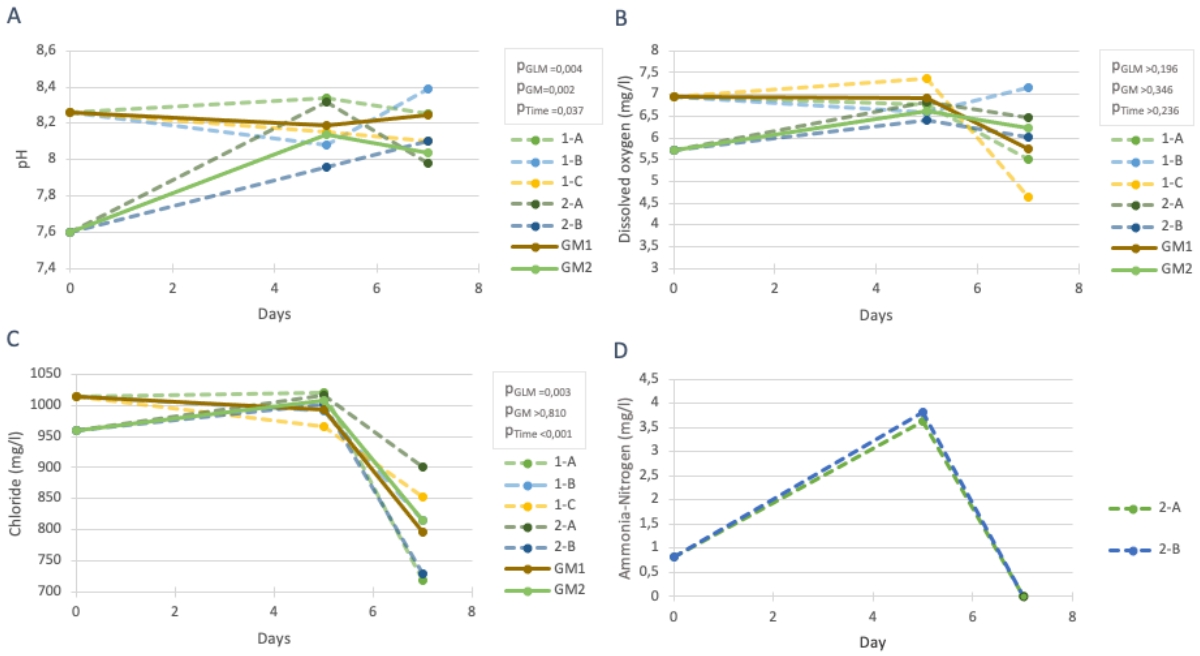

Figure 17 – Changes in water parameters in water samples extracted at day 0, 5 and 7 of the experiment for growth media 1 (GM1, n=3) and growth media 2 (GM2, n=2). All replicates represented in addition to the average of the growth media (GM). Figure A show the change in pH. Figure B show the change in dissolved oxygen. Figure C show the change in chloride and figure D show development of ammonia (NH_3-N) in water samples of GM2. For NH_3-N data was only available for GM2. The two replicates are presented. Statistical analyses were conducted using general linear model (GLM) selecting GM and time as fixed factors. Differences were found for pH, chloride, and ammonia.

For water temperature all samples ranged between 17,9°C and 20,3°C. For pH, DO and Cl no difference was seen between replicates within GM1 and GM2 (p>>0,05). Between growth media, a significant difference was seen in pH during the 7 days (p=0,010). The average pH value for GM1 was 8,3±0,0 on day 0 of the experiment, and 7,6±0,0 in GM2. For day 5 the numbers were 8,2±0,13 and 8,1±0,26, and for day 7, 8,2±0,15 and 8,0±0,08, for GM1 and GM2, respectively. Figure 17-A show the trends in pH development based on water samples collected during the experiment, showing significant differences as a function of time (p=0,037) and for the factor growth media (p=0,002).

For the water parameter, DO, no significant difference was seen between the growth media during the experiment (p>0,405). The average content for GM1 was 6,93±0,0 mg/l on day 0 of the experiment, and 5,73±0,0 mg/l in GM2. For day 5 the content was 5,91±0,41 mg/l and 6,62±0,28 mg/l, and for day 7, 5,76±1,27 mg/l and 6,24±0,32 mg/l, for GM1 and GM2, respectively. Figure 17-B show the development in DO content during the experiment

For Cl content, no significant difference was seen between growth media (p>0,905). The average Cl content for GM1 was 1015,0±0,0 mg/l on day 0 of the experiment, and 959,0±0,0

mg/l in GM2. For GM1 it decreased to 992,67±27,54 mg/l on day 5, while in GM2 it increased to 1008,50±10,61 mg/l. On day 7 average measurements were and 795,67±69,06 mg/l and 815,0±120,21 mg/l for GM1 and GM2, respectively. Figure 17-C show the trends in Cl development based on water samples collected during the experiment, showing significant differences as a function of time ($p<0,001$), but not for the factor growth media ($p<0,810$). The level of NH_3-N was too low for detection in all samples of GM1 and was shown as <001mg/l (Appendix 7). Results for GM2 are shown in Figure 17-D. NH_3-N content starts low at 0,82 mg/l before it increases to 6,82 mg/l for 2-A and 6,42 mg/l for 2-B at day 5 and decrease to a non-detectable amount at day 7 (<0,01 mg/l). To see how different water parameters in the sample could affect each other, Pearson correlation was performed at a 95% confidence interval (CI) (Appendix 8). The result shows a strong correlation between NH_3-N and Cl (0,97, $p<0,032$, 95% CI).

4.2.3 Water Samples Analyzed at Eurofins

Water from Osan smolt facility used in the experiment was analyzed by Eurofins at the start and end of the project. 'Water input' is the same water that has been analyzed at NTNU's Food Science laboratory (day 0). Fe, Cu, pH, Zn and SS were not analyzed for the water at the end of the experiment.

The results (Table 5) show removal of 10,8% of total P and 3,9% of total N for GM1, and a smaller uptake for GM2 with 1% and 0%, respectively. Other nutrients decreased over the 7 days in GM1 and GM2 accordingly: NH_4-N with 94,8% and 99,58%, NO_3-N and NO_2-N with 5,4% and 32,35%, Ca with 2% and 9,09% and K with 27,5% and 7,69%. PO_4-P decreased in GM1 by 33,8%, while it increased in GM2 by 12,86%. The concentration of Mg did not change in GM1 but increased by 3,57% in GM2.

Table 5 – Results from the water analysis of water inputs and outputs of the experiment, performed by Eurofins. Values are given in mg/l and decrease in percent. Negative values in percent indicate increase in nutrient content, while minus alone represent missing data points. SS stands for suspended solids.

	pH	SS	Total P	PO_4-P	Total N	(NO_3+NO_2)-N	NH_4-N	K	Ca	Cu	Mg
Growth media 1											
Input (mg/l)	7,6	6,7	7,4	7,4	51	37	0,31	12	100	0,004	5,2
Output (mg/l)	-	-	6,6	4,9	49	35	0,016	8,7	98	-	5,2
Decrease (%)	-	-	10,8	33,8	3,9	5,4	94,8	27,5	2	-	0
Growth media 2											
Input (mg/l)	6,8	62	10	7	53	34	4,5	13	110	0,007	5,6
Output (mg/l)	-	-	9,9	7,9	53	23	0,019	12	100	-	5,8
Decrease (%)	-	-	1	-12,86	0	32,35	99,58	7,69	9,09	-	-3,57

4.2.4 Plant Growth

The biomass production of *Lactuca sativa* was assessed. Results are provided on fresh mass, dry mass and ash. Visual observations and pictures are included to give a better impression of the overall plant growth.

Weight and Height

Parameters for weight and height development during the experiment are presented as the average and standard deviations (x̄±SD) for each replicate in Table 6. P-value is used to see if there is any significant difference within the different growth media. Due to the experimental design the repeatability of CP was not possible to test.

Table 6 – The table show the different plant parameters regarding height and weight measured during the experiment in growth media 1 (n=27), growth media 2 (n=18) and control plants (n=12). Statistical analyses were conducted using one-way ANOVA selecting replicates as factor. Results are presented as average of the replicates, with standard deviation (x̄±SD) and p-values. Minus represent missing data points.

Growth media	**Growth media 1**				**Growth media 2**			**Control plants**
Replicate (x̄±SD)	**1-A**	**1-B**	**1-C**	**P-value[1]**	**2-A**	**2-B**	**P-value[1]**	**CP**
Start height (mm)	66,2 ± 5,2	66,0 ± 10,7	64,4 ± 7,0	0,859	64,3 ± 9,3	66,8 ± 10,8	0,614	72,0 ± 10,6
End height (mm)	97,0 ± 20,9	94,4 ± 9,6	90,8 ± 9,9	0,663	98,1 ± 18,6	93,8 ± 10,7	0,553	106,8 ± 23,7
Daily growth rate (mm)	4,4 ± 2,9	4,1 ± 0,9	3,7 ± 1,4	0,788	4,8 ± 1,9	3,9 ± 0,9	0,186	5,0 ± 2,3
Change in height (%)	46,8 ± 30,2	44,8 ± 15,2	41,8 ± 17,6		52,6 ± 18,9	41,9 ± 14,4		48,0 ± 18,1
Start weight (g)	4,4 ± 2,2	5,2 ± 2,1	5,5 ± 2,0	0,518	5,3 ± 2,3	6,2 ± 1,9	0,379	-
End weight (g)	11,6 ± 3,5	10,5 ± 3,2	11,3 ± 2,8	0,753	12,6 ± 3,0	12,0 ± 2,5	0,616	-
Daily growth rate (g)	1,0 ± 0,4	0,8 ± 0,3	0,8 ± 0,2	0,172	1,0 ± 0,2	0,8 ± 0,3	0,112	-
Change in weight (%)	197,0 ± 106,0	121,0 ± 65,6	122,4 ± 62,5		170,7 ± 98,3	120,1 ± 115,3		-

[1]P-values are found for differences within the growth media, based on the average and standard deviations of the replicates (x̄±SD). CP is excluded from weight parameters as roots were in soil.

For height, no significant differences could be seen within the growth media, at the start or end of the experiment ($p>>0,05$) (Table 6). Plants grew during the experiment, giving significant differences between start and end height, within the different growth media (Figure 18-A) ($p<0,001$). Between growth media no significant difference was seen for height ($p>>0,05$). Average values for plant growth in height are seen in Figure 18. The average daily growth rate in soil was 4,98±2,33 mm, while for hydroponic plants in GM1 and GM2 it was 4,07±1,89 mm and 4,34±1,52 mm, respectively.

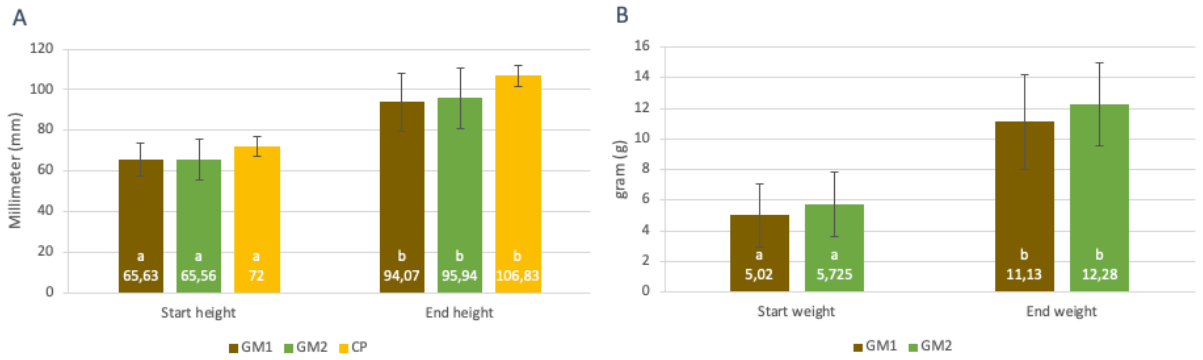

Figure 18 – Figure A show difference in start (p>0,106) and end height (p>0,092), for all growth media during the experiment, growth media 1 (GM1, n=27), growth media 2 (GM2, n=18) and control plants (CP, n=12). Figure B show differences in start (p>0,508) and end weight (p>0,416) between the hydroponic growth media (GM1, n=27 and GM2, n=18). Figures are presented as average of the growth media, with standard deviation (x̄±SD). Statistical analyses were conducted using one-way ANOVA selecting growth media as factor. Different letters show significant differences (p<0,05) between the groups, at the start and the end of the experiment. Control plants (CP) are not included in weight parameters.

For the parameter plant weight no significant differences were seen within growth media at the start or end of the experiment (Table 6). CP were excluded from weight parameters. No significant difference was seen between the two hydroponic growth media at the start (p>0,508) and end of the experiment (p>0,416). Plants grew significantly during the experiment for both GM (p<0,001), which is shown in Figure 18-B. Average daily growth rates in weight for GM2 and GM1 was 0,87±0,30g and 0,94±0,27g, respectively.

Leaf Formation and Visual Registrations

The number of leaves at the start and at the end were registered for all systems and are listed in Table 7. Leaves are presented with p-values which shows that plants within the growth media had no significant difference (p>>0,05).

Table 7 – Results from registrations on leaves in the start and end of the experiment, in addition to new leaves developed during the 7 days of experiment, in growth media 1 (n=27), growth media 2 (n=18) and control plants (n=12). Statistical analyses were conducted using one-way ANOVA selecting replicates as factor. Results are presented as average of the replicates, with standard deviation (x̄±SD) and p-values.

Growth media	**Growth media 1**				**Growth media 2**			**Control plants**
Replicate (x̄±SD)	**1-A**	**1-B**	**1-C**	**P-value[1]**	**2-A**	**2-B**	**P-value[1]**	**CP**
Start leaves	4,33 ± 0,52	4,11 ± 0,33	4,11 ± 0,33	0,404	4,11 ± 0,33	4,00 ± 0,0	0,332	3,75 ± 0,62
End leaves	7,50 ± 0,93	6,78 ± 1,09	7,33 ± 0,50	0,164	7,60 ± 0,50	7,20 ± 0,40	0,165	6,58 ± 1,08
New leaves	3,10 ± 1,10	2,70 ± 0,90	3,20 ± 0,40	0,291	3,40 ± 0,50	3,20 ± 0,40	0,346	2,80 ± 0,80

[1]P-values are found for differences within the growth media, based on the average and standard deviations of the replicates (x̄±SD). Control plants (CP) consisted of one replicate, and the p-value was not found.

All plants started with approximately 4 leaves when placed in the systems. Still, a significant difference was seen between start leaves in the different growth media (p=0,014). GM1, GM2 and CP had an average of 4,19±0,40, 4,06±0,24 and 3,75±0,62 start leaves, respectively. Figure 19-A show that the difference is found between GM1 and CP. A significant difference was also seen at the end of the experiment (p=0,035), where GM1, GM2 and CP had an average of 7,22±0,90, 7,39±0,50 and 6,58±1,09 end leaves, respectively. Figure 19-B shows that the difference was found between GM2 and CP. The development of new leaves showed no significant difference between any of the growth media (p>0,192), with the average of 3,04±0,85, 3,33±0,48 and 2,83±0,84 new leaves for GM1, GM2 and CP, respectively.

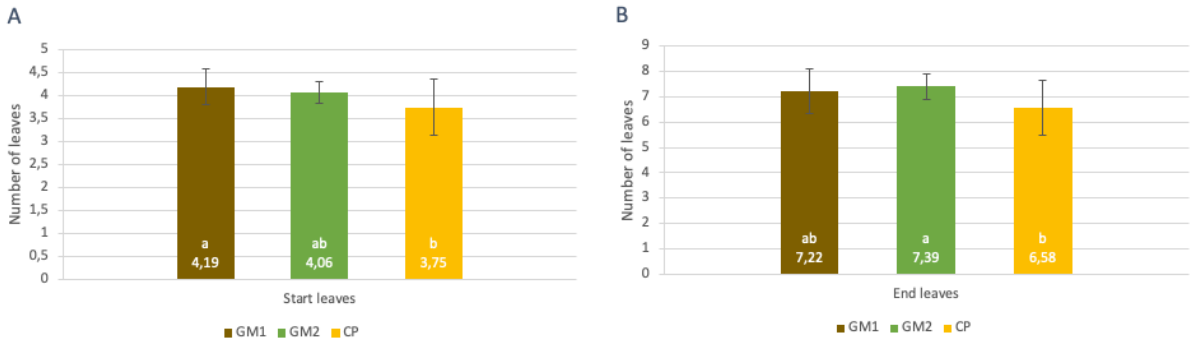

Figure 19 - Figure A show the average start leaves at the beginning of the experiment for growth media 1 (GM1, n=27), growth media 2 (GM2, n= 18) and control plants (CP, n=12) (p=0,014). Figure B show the average amount of leaves on day 7 for the same growth media (p=0,035). Figures are presented as average of the growth media, with standard deviation ($\bar{x}$±SD). Statistical analyses were conducted using one-way ANOVA selecting growth media as factor. Different letters show significant differences (p<0,05) between GM1 and CP for start leaves and differences between GM2 and CP in end leaves.

Visual observations show hydroponic plants as green, fresh, crispy and compact. No obvious difference was spotted between the hydroponic plants. Soil plants had a lighter green color on the leaves, were less crispy and leaves were longer and hanging more compared to hydroponic plants. Figure 20 show plants grown hydroponically and in soil.

Figure 20 – Pictures to the left show plants grown in water from after the biofilter (1-C) at the end of the experiment. Pictures to the right show control plants (CP) in soil plants at day 5.

Difference in growth during the 7 days can be seen in Figure 21.

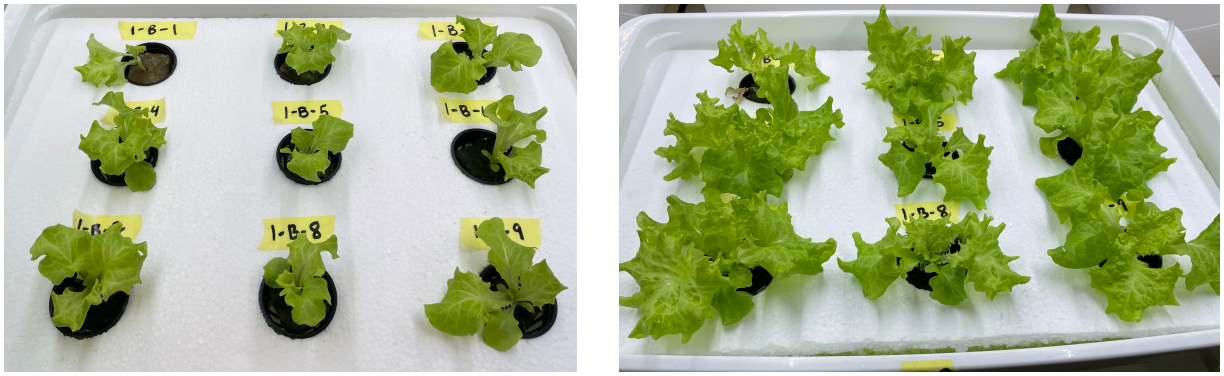

Figure 21 - Picture to the left shows the plants in replicate 1-B on day 0, pictures to the left show the same plants on day 7.

Dry Matter and Ash Content

Dry matter and ash content was analyzed to allow physiological comparison. Results are presented as dry matter (DM) and ash content (AC) in percent of fresh weight (FW), to correspond to presentations of results in nutrient uptake. Within the growth media, no significant differences were seen for DM. For AC significant differences were seen between replicate 1-A and 1-C in GM1, and no differences in GM2 (Table 8).

Table 8 - Dry matter and ash content of plants in percent, after drying and burning lettuce samples in growth media 1 (1-A n=9, 1-B n=9, 1-C n=9 and growth media 2 (2-A n=9, 2-B n=9 (dry matter) and n=7 (ash content) and control plants (n=12). Results are presented as average of the replicates, with standard deviation (x̄±SD) and p-values.

Growth media	**Growth media 1**				**Growth media 2**			**Control plants**
Replicate (x̄±SD)	**1-A**	**1-B**	**1-C**	**P-value[1]**	**2-A**	**2-B**	**P-value[1]**	**CP**
Dry matter (%)	5,10 ± 0,65	5,04± 0,27	5,03 ± 0,14	0,936	5,10 ± 0,22	5,14 ± 0,63	0,738	4,52 ± 0,21
Ash content (%)	0,99 ± 0,10[a]	1,10 ± 0,10[ab]	1,10 ± 0,04[b]	0,028	1,02 ± 0,04	1,17 ± 0,39	0,265	1,10 ± 0,10

Dry matter (DM) and ach content (AC) calculated based on fresh weight of lettuce. [1]P-values are found for differences within the growth media (GM), based on the average and standard deviations of the replicates (x̄±SD). Letters represent significant differences ($p<0,05$) for AC in GM1, between 1-A and 1-C.

Dry matter accounted for an average of 5,06±0,4% for plants in GM1 and 5,10±0,43% in GM2, while for soil plants (CP) it accounted for 4,52±0,21%. There was a significant difference between the growth media ($p<0,001$). The difference was found between hydroponic and soil plants, as seen in Figure 22-A. The ash content accounted for an average of 1,05±0,8% for plants in GM1, 1,09±0,26% in GM2 and in CP it accounted for 1,09±0,05%, of FW. No significant difference was seen between the growth media ($p>0,566$), as shown in Figure 21-B. Of DM, the AC accounted for 20,48±1,92% in GM1, 19,62±1,26% in GM2 and 23,97±0,55% in CP.

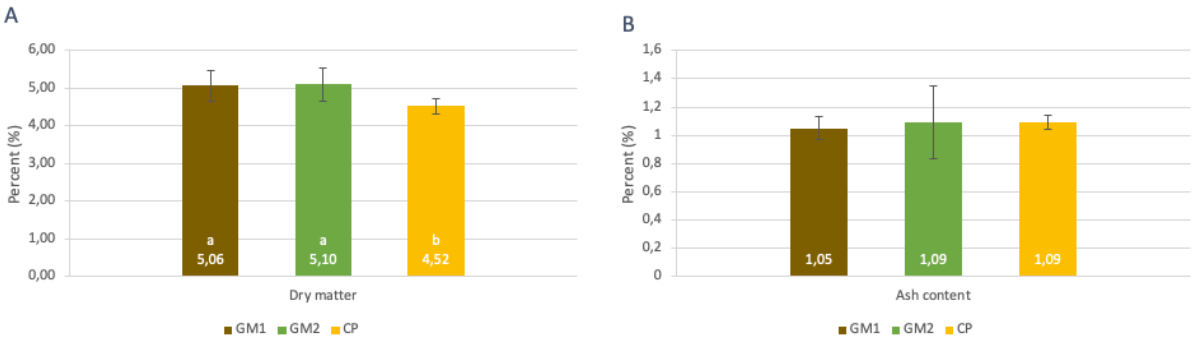

Figure 22 – Figure A show the average dry matter ($p<0,001$) in percent of fresh weight for growth media 1 (GM1, n=27), growth media 2 (GM2, n= 18) and control plants (CP, n=12). Figure B show the average ash content ($p>0,566$) in percent of fresh weight, for the different growth media (GM1, n=27, GM2, n=16, CP, n=12). Figures are presented as average of the growth media, with standard deviation ($\bar{x}\pm SD$). Statistical analyses were conducted using one-way ANOVA selecting growth media as factor. The letters show significant differences ($p<0,05$), which occurred between hydroponic growth media (GM1 and GM2), and control plants (CP) in soil, for dry matter.

4.2.5 Calculations on Nutrient Uptake

NO_3^- in the produced plant biomass is shown in Table 9. Plants grown in water from after biofilter (GM1) absorbed on average 112,2 mg NO_3^-, based on calculations that NO_3^- accounts for 4% of dry matter (Maynard et al., 1976). For plants grown in water from the plate separator (GM2), the number is slightly higher at 113,5 mg. For NO_3^- content to relate with nutrients removed from system, NO_3-N is listed as well.

Table 9 - Calculations on nitrate (NO_3^-) in the produced biomass based on dry matter (DM) in lettuce plants.

Growth media	**Growth media 1**			**Growth media 1**		**Control plants**
Replicate	1-A	1-B	1-C	2-A	2-B	CP
Δ Change in biomass (g)	65,0	48,0	52,0	66	52	54,3
Average DM (%)	5,1	5,1	5,1	5,1	5,2	4,5
DM biomass (9 plants)	3,3	2,5	2,7	3,4	2,7	2,4
Nitrate produced (g)[1]	0,1	0,1	0,1	0,1	0,1	0,1
Nitrate produced (mg)[1]	132,6	97,9	106,1	134,6	108,2	97,7
Average per GM (mg)		112,2			113,5	97,7
Average per plant (mg)		12,5			12,6	10,9
NO_3-N per GM (mg)[2]		25,4			25,7	22,1
NO_3-N per plant (mg)[2]		2,8			2,9	2,5

[1] NO_3^- is 4% of DM (Maynard et al., 1976). [2] Nitrate nitrogen (NO_3-N) is found related to the total mass of N in NO_3. Equations 6 and 7 are used to calculate the given values (section 3.4).

Eq. 6: $DM\ (g) = \frac{\bar{x}DM\ (\%)\ x\ D\ (g)}{100}$

DM – Dry matter biomass (9 plants)
$\bar{x}$DM – Average dry matter per growth media, in percent
D – Change in biomass (end weight – start weight

Eq. 7: $NP\ (g) = \frac{NDM\ (\%)\ x\ DMPB\ (g)}{100}$

NP - Nutrients produced
NDM - Nutrient concentration in dry matter
DMPB – Dry matter in produced biomass.

Performing the same calculations on total P, accounting for 0,2% of DM (Estrada et al., 2016), results for GM1 and GM2 are 5,6 mg and 5,7 mg, respectively (Table 10).

Table 10 Calculations on phosphorus (P) in the produced biomass based on dry matter (DM) in lettuce plants

Growth media	**Growth media 1**			**Growth media 2**		**Control plants**
Replicate	1-A	1-B	1-C	2-A	2-B	CP
Δ Change in biomass (g)	65,0	48,0	52,0	66	52	54,3
Average DM (%)	5,1	5,1	5,1	5,1	5,2	4,5
DM biomass (9 plants)	3,3	2,5	2,7	3,4	2,7	2,4
Phosphorus produced (g)*	0,0	0,0	0,0	0,0	0,0	0
Phosphorus produced (mg)*	6,6	4,9	5,3	6,7	5,4	4,9
Average per GM (mg)		5,6		5,7		4,9
Average per plant (mg)		0,6		0,6		0,5

[1]P is 0,2% of DM (Estrada et al., 2016). Equations 6 and 7 are used to calculate the given values (section 3.4).

Eq. 6: $DM\ (g) = \frac{\bar{x}DM\ (\%)\ x\ D\ (g)}{100}$

DM – Dry matter biomass (9 plants)
$\bar{x}$DM – Average dry matter per growth media, in percent
D *– Change in biomass (end* $weight - start\ weight$*)*

Eq. 7: $NP\ (g) = \frac{NDM\ (\%)\ x\ DMPB\ (g)}{100}$

NP - Nutrients produced
NDM - Nutrient concentration in dry matter
DMPB – Dry matter in produced biomass.

Nutrients removed from each system (mg/l) multiplied by volume (35l), show approximately the total nutrient removal for each of the systems. Dividing it by 9 gives a rough idea of how much each plant ideally absorbed. Table 11 and Table 12 show the removal in GM1 and GM2, respectively.

Table 11 – Nutrient uptake by lettuce plants grown in water from after biofilter (GM1 in RAS), based on water samples by Eurofins. 9 plants / 35l RAS water. All values in mg/l and mg.

Growth media 1	**Removed from system (mg/l)**	**Removed per container (35l) (mg)**	**Per plant (mg)**
Total N	2,0	70,0	7,8
(NO_3+NO_2)-N	2,0	70,0	7,8
NH_4-N	0,3	10,5	1,2
Total P	0,8	28,0	3,1
PO_4-P	2,5	87,5	9,7
K	3,3	115,5	12,8
Ca	2,0	70,0	7,8

Equation 8 is used to calculate the given values (section 3.4).

Eq. 8: $NR\ (mg) = DN(mg/l)x\ V(l)$

NR – Nutrient removal from the system (mg)
DN *– Change in nutrient content (mg/l)*
V – Volume of system (l)

Table 12 - Nutrient uptake by lettuce plants grown in water from the plate separator (GM2) in RAS, based on water analysis by Eurofins. 9 plants / 35l RAS water. All values in mg/l and mg.

Growth media 2	Removed from system (mg/l)	Removed per container (35l) (mg)	Per plant (mg)
Total N	0,0	0,0	0,0
(NO_3+NO_2)-N	11,0	385,0	42,8
NH_4-N	4,5	157,5	17,5
Total P	0,1	3,5	0,4
PO_4-P	0,9	-	-
K	1,0	35,0	3,9
Ca	10,0	350,0	38,9

Equation 8 is used to calculate the given values (section 3.4).

Eq. 8: $NR\ (mg) = DN(mg/l) x\ V(l)$

NR – Nutrient removal from the system (mg)
$DN - Change\ in\ nutrient\ content\ (mg/l)$
V – Volume of system (l)

In 7 days, 70 mg of the total N in water from after biofilter (GM1) was removed from the system, ideally meaning 7,8 mg per plant. No uptake was seen for total N in water from the plate separator (GM2). For total P the numbers for GM1 and GM2 were 28 mg/system and 3,1 mg/plant, and 3,5 mg/system and 0,4 mg/plant, respectively. Looking closer into N and P compounds show a removal rate per plant of 7,8 mg and 42,8 mg NO_3-N and NO_2-N, 1,2 mg and 17,5 mg NH_4-N, and 9,7 mg and an increase for PO_4-P, in GM1 and GM2, respectively.

4.3 Water Samples from a Norwegian Smolt Facility

Results from water samples collected at the Norwegian smolt facility (anonymized) are presented in Table 13. Values show a small decrease in total P (from 2 mg/l to 1,8 mg/l) and total N (from 17 mg/l to 16 mg/l) in water after passing the biofilter in RAS. Similar trends are seen for the other nutrients. No change is seen for NO_3-N and NO_2-N.

Table 13 – Results from water analysis of RAS water from a Norwegian smolt facility (anonymized), collected after mechanic filtration and after biofilter. The analysis is performed by Eurofins and all values are given in mg/l. Explanation of all abbreviations are seen on page ix.

	pH	SS	Total P	PO_4-P	Total N	(NO_3+NO_2)-N	NH_4-N	K	Ca	Cu	Mg
After mechanic filter	6,7	2,5	2	1,8	17	15	0,61	3,1	27	0,0012	1,4
After biofilter	6,9	2	1,8	1,7	16	15	0,18	2,8	26	0,0011	1,4

5 Discussion

The present research contributes to fill an information gap regarding aquaponics in combination with Atlantic salmon production in RAS. Finding data on the specific topic was challenging, as this is a newer area of research. Several actors were contacted during the literature review, stating that the problem statement is highly relevant at this point in time (Appendix 3). The industry is currently researching similar questions, to find a way to integrate a plant section and create more sustainable food production. Much data can be found on aquaponics and warm water species, but there are few publications on cold-water species such as Atlantic salmon.

5.1 Exploring the Potential of Plant Growth in Combination with RAS

The literature study shows a potential for plant growth combined with RAS. Cifuentes-Torres et al. (2021) state that aquaponics can be used to eliminate dissolved N and P from aquaculture systems, as plants absorb the nutrients through the roots and utilize them for plant growth. An increased focus on aquaponics in relation to land-based aquaculture is therefore seen, and several actors are planning to build large-scale commercial systems combining fish production in RAS, with plant production (Columbi Salmon, Superior Fresh, Smart Salmon, Greenaquanor). Greenaquanor is planning an aquaponics system producing up to 90 tons of trout and 567 tons of greens and berries. The filtering efficiency of the integrated RAS is expected to be 100% of N and 100% of P, meaning zero release of the nutrients into the environment (Hilmarsen, 2019).

Jakobsen et al. (2021) state that the potential for using dissolved nutrients from aquaculture for plant cultivation is considerable. High levels of NO_3^- are accumulated when plants are grown in soilless solutions (Boon et al., 1990), and NO_3^- is one of the nutrients that must be removed from RAS. Hydroponic nutrient solutions commercially used consist of NH_4^+, NO_3^-, or even NH_2·, dependent on the pH in the root environment (Sonneveld & Voogt, 2009a). These compounds correlates to the nutrients Brod (2021), Anderson et al. (2019) and Rakocy et al. (2006) reports in aquaculture. The nutrient concentrations found in aquaculture are much lower than in commercial nutrient solutions, as pointed out by CIRiS (2020). In aquaponics with the right fish stocking (coupled system/closed loop), NO_3^- levels are high enough for plant growth, but levels of K and P are insufficient for optimal growth. Ca and Fe can also be insufficient, which could limit the growth (Maucieri et al., 2019). Extra nutrients, especially K and P should

be added to support the efficient reuse of nutrients (Maucieri et al., 2019; Nicoletto et al., 2018). Furlani et al. (1999) and Domingues et al. (2012) state that the nutrient absorption in hydroponic plants is often proportional to the nutrient concentration in the solution surrounding the roots. The uptake is influenced by conditions such as oxygenation, temperature, salinity, EC, pH, light intensity, air humidity and photoperiod. For the plant section to get sufficient nutrients, large amounts of water must pass through the system (CIRiS, 2020).

The amount of water available for plant production varies according to the water exchange rate of the system. For the Norwegian smolt facility (anonymized), 2% new water is added to the system, meaning 2% is also removed every day. One grow-out section has a water intake of 630,72 m^3/day, meaning a large quantity of water is available for plant growth. Boogaard is a water quality specialist and informs that the content of nutrients in RAS facilities varies considerably over the production period and follows the feeding trend (Boogaard, M., Personal Communication, October 5th, 2021). He says smolt facilities do not have a stable nutrient level in the operating water, which is important to consider for the hydroponic part of the system.

Structuring a RAS with hydroponics can be done in different ways, depending on the priorities of the system. Building decoupled systems on a side stream of water from RAS, like Jakobsen et al. (2021) tested, may be a better option than a closed loop. Including a water control system where EC and pH can be lowered may improve growth. Jakobsen et al. (2021) state that these parameters are optimal for fish, but can limit growth in plants. Goddek, Joyce, Wuertz, et al. (2019) states that nutrients can be added to improve the nutrient content in RAS water and achieve better plant growth. Decoupled systems are also Columbi Salmons preferred production method for the aquaponic system they plan to build in Belgium (Columbi Farms, 2022). A common view found through the literature review is that when including hydroponics in commercial RAS, the focus is on the fish, and the plant growth must not negatively affect the fish.

5.2 Plant Experiment

A plant experiment was performed to generate data on nutrient uptake and plant growth under conditions similar to RAS combined with hydroponics. Water from RAS was extracted from two possible outtake points at Osan smolt facility, based on preliminary research of nutrient content in RAS and discussions with the operation manager (Øren, S. O., Personal

communication, January 21, 2022). Using water from the plate separator was not the preferred choice, but a backup as it was not possible to get wastewater from any facilities close to the location of the experiment. After more research, it showed to be a good choice, as wastewater from Osan might be less suitable for plant production due to the zero-water exchange system, which will be further discussed below.

When collecting the water from the RAS it had a low temperature (12°C). It was therefore left over night to adjust to the room temperature. The following day, temperatures had reached 21°C and the plants could be transferred. The room temperature during the experiment varied, due to people going in and out of the room, allowing cool air to enter. This may have created a small variation in the water temperature.

Replicate 2-C (shown in Figure 12) was excluded from all results due to a leak after the air hose disconnected from the pump. The tank was refilled, but this simultaneously replenished the tank with nutrients. 2-C could therefore not be seen as a replicate, and was removed. Air hoses should be more securely fastened to avoid leaks in future experiments. Adding more replicates to the experimental design could improve the results of this experiment. Having only 3 replicates originally made the design vulnerable, as seen when 2-C had to be removed.

5.2.1 Physiochemical Water Parameters During the Experiment

Physiochemical water parameters were controlled during the experiment and extracted water samples were analyzed at the laboratory. The water temperature during the experiment was not kept at a stable level and increased over the 7 days, yet all measured temperatures were within ranges (18-26°C) suggested by Jenco (2019)

EC developed differently between the growth media as a function of time, as seen in Figure 16-B. GM2 had a significantly higher EC than GM1. The difference in SS may be of importance, as SS in GM2 was as high as 62 mg/l, while in GM1 it was 6,7 mg/l. Still, the EC ranged between 3,98 to 4,59 mS/cm for all replicates throughout the experiment. This is contrary to the findings from Hess-Erga et al. (2013), stating an EC between 0,3-0,6 mS/cm in aquaponics. It was also higher than EC (0,7-0,8 mS/cm) seen in research by Delaide et al. (2016). Hess-Erga et al. (2013) recommend values between 1,5-3,5 mS/cm for plant growth in hydroponic systems, while Morgan (1999) specifies values of 2-2,5 mS/cm for lettuce. The values in the

system was higher than recommended in literature. It was expected that the EC would decrease as the plants absorbed nutrients, but no clear trend was observed. Ding et al. (2018) state that high EC values can affect nutrient uptake in plants and lead to lower biomass production.

The pH during the experiment was above 7,5 and below 8 for all replicates and no significant differences were seen between growth media. Delaide et al. (2016) state that for lettuce production the pH range should be between 6,0 to 8,0 to secure the uptake of macronutrients. According to Anderson et al. (2019) the pH in aquaponics systems is usually around 7,0, which corresponds to the pH reported in production water at Osan (section 3.3).

The pH increased from the time water was collected to the start of the experiment. Still, the pH values were within the limit for lettuce production, but higher than what is seen in closed-loop aquaponics (Anderson et al., 2019). In an experiment by Delaide et al. (2016) the pH ranged between 7,10 to 7,94, showing similar pH levels as in this experiment. The pH increased at the start of the experiment. The reason for this is unknown, but Graber and Junge (2009) point out that pH in aquaponics systems often increases due to the nitrification process. Different bacteria could be present in the unsterilized water from RAS, including nitrifying bacteria. Water samples analyzed at the laboratory show different pH values for GM1, compared to the pH measured during the experiment (8,3 and 7,7, respectively). It shows a significant difference between the hydroponic growth media, even though water samples were frozen immediately after extraction and directly measured when thawed. pH measured during the experiment showed no significant differences between the hydroponic growth media.

The DO developed differently between the growth media as a function of time, as seen in Figure 16-D. Yet, no significant difference was seen between the growth media. After day 0, all replicates were connected to air pumps providing roughly the same amount of oxygen. Levels during the experiment ranged from 6,8 to 8,5 mg/l, showing similar results as Delaide et al. (2016) who had an average DO of 7,36 mg/l during their lettuce trial in aquaponics. These levels are higher than what Maucieri et al. (2019) recommend to avoid low uptake performance by roots (4-5 mg/l). Baras (2018) states that high oxygen levels can lead to stunned growth and less biomass, but when using air pumps as the only source of oxygen, it will not be possible to reach levels high enough to affect plants. Indicating that the plants had enough DO, and still levels were not too high.

For Cl, no differences were seen between the growth media and there was an overall decrease in Cl content, which is seen in Figure 17-C. A similar pattern of results was obtained by Jakobsen et al. (2021), showing a decrease in Cl content for all replicates in a simulation of aquaponics. This is supported by Yang and Kim (2020) findings that plants in aquaponics accumulated higher levels of Cl than other plants. Cl is a macronutrient and is required in relatively large amounts (Yang & Kim, 2020).

Results for NH_3-N could only be registered in GM2, as the levels were too low for detection in GM1. GM2 shows an increase in NH_3-N before it decreases to undetectable levels. This observation could not be explained. The probe used measured levels of NH_3-N, which were very low. Measuring TAN and then calculating the amount of NH_3-N based on pH, could have been a better method to obtain data and get more accurate readings than <0,001mg/l. A correlation was found between NH_3 and Cl, but no direct relationship has been stated in previous literature found on aquaponics.

5.2.2 Nutrient Uptake

Comparing uptake of NO_3^-, NH_4^+, and PO_4^{3-} to uptake of total N and P give rise to questions. How can higher amounts of NO_3^-, NH_4^+, and PO_4^{3-} be removed, when the decrease in total N and P is so low? This difference may have occurred due to analysis methods used at Eurofins, as NO_3^-, NH_4^+, and PO_4^{3-} were analyzed by spectrophotometry (DA) and total N and P by spectrophotometry (CFA). In the following discussion results from spectrophotometry (DA) are considered more accurate. Plant nutrient uptake was lower than expected, with NH_4^+ being the most efficiently absorbed nutrient. Low removal of N and P may indicate that plants do not efficiently absorb the nutrients under the conditions given in the experiment.

Available NO_3-N and NO_2-N during the start of the experiment were 37 mg/l and 34 mg/l for GM1 and GM2, respectively. These numbers are similar to NO_3-N levels found in aquaponics by Delaide et al. (2016). Comparing these results with the nutrient solution made by Hoagland shows large differences. Hoagland suggests 200 mg/l NO_3-N and NO_2-N in an optimal nutrient solution, while Resh (2022) states even higher levels (730 mg/l) for optimal hydroponic lettuce production. Both reported far higher values of NO_3-N than what was available in the RAS water. NO_3-N and NO_2-N were removed by 5,4% in GM1 and 32,35% in GM2, which is much lower than the findings by Endut et al. (2011). In an experiment with water spinach in

combination with African catfish, they found an uptake rate of 82,93–92,22% and 79,17–87,10% for NO_2-N and NO_3-N, respectively. These results correspond with findings from Lam et al. (2015) for spinach combined with marble goby fish (87% NO_2-N and 70% NO_3-N, respectively). The findings are not directly comparable as both different fish and plant species were used, but it gives some indications of nutrient uptake rates in aquaponics.

Santamaria (2005) states that NO_3^- is the main source of N for lettuce. Calculations on N compounds are therefore limited to NO_3-N. Results show that the nutrient was present in much higher concentrations than what was removed during the 7 days, as seen in Table 5. The calculated NO_3-N content in the lettuce (25,36 mg in GM1, 25,65 mg in GM2, and 22,09 in CP) does not directly correspond with the amount removed from the system (70 mg in GM1 and 385 mg in GM2). The amount of nutrients removed from the system is much higher than expected accumulated in plants, based on calculations. It must be mentioned that the amount of NO_3-N removed from the system is combined with NO_2-N, but this is not expected to account for a large portion as it is dangerous for fish and only present in very small amounts (Appendix 2). In GM1 7,8 mg/plant of NO_3-N and NO_2-N were removed from the system, while the number was 42,8 mg/plant in GM2. The NO_3-N in the produced biomass in one plant should, based on calculations, account for 2,82 mg on average for GM1, 2,85 mg for GM2, and 2,45 mg for CP, which is much lower than suggested by Hoagland at 855,5 mg/plant.

GM1 shows a higher removal of total N than GM2, while GM2 shows a higher removal for NH_4^+, and NO_3-N and NO_2-N. The pH was above 7,0 for all replicates, indicating more alkaline environments. Delaide et al. (2016) state that lettuce preferred form for uptake at higher pH is NH_4^+, which corresponds to the pH in the system and the high uptake of NH_4^+, but low uptake of NO_3^-.

Total P in the water during the experiment was 7,4 mg/l and 10 mg/l for GM1 and GM2, respectively. For PO_4^{3-} the numbers were similar at 7,4 mg/l and 7 mg/l. Compared to the nutrient solution suggested by Hoagland (24 mg/l), these numbers are much lower. Total P was removed by 10,8% and 1% for GM1 and GM2, respectively, during the experiment. This is low, as findings by Eck et al. (2019) indicate that all P available in the production water can be absorbed by plants. Lam et al. (2015) saw a 60% removal rate. Because of this large difference in removal, the suitability of the analysis methods at Eurofins is again questioned. Eck et al. (2019) state that the uptake rate depends on the design of the system. P is often the limiting

factor for growth in aquaponics (Graber & Junge, 2009; Seawright et al., 1998), but water analysis shows the remaining amounts of total P in the output water. This indicates that the P was not available for plants, or that the plants were not in the system long enough to absorb it. GM1 shows a higher removal of total P than GM2, with a difference of 9,8%. At the same time, PO_4^3-P decreased in GM1, but increased in GM2 (12,8%). The increase is opposite to results found by Endut et al. (2011) who saw the removal of 75,36–84,94% of PO_4^{3-}, which accounts for an average of 26% PO_4-P [1].

P removed from the systems during the experiment was 3,1 mg/plant and 0,4 mg/plant for GM1 and GM2, respectively, while the total P in the produced biomass of one plant should, based on calculations, account for 0,62 mg on average for GM1, 0,63 mg for GM2, and 0,54 mg for CP. Hoagland's suggestion indicates 93,3 mg/plant, being much higher than what was available during the experiment. Still, calculations suggest that more P was removed from GM1, than expected accumulated in plants. Whether plants absorbed this P, is not known.

Delaide et al. (2016) inform that pH can influence the uptake of P and that a pH of 6,0-8,0 is recommended. As shown previously, the pH in the system ranged between 7,5-7,96 (Figure 16). Yavuzcan Yildiz et al. (2017) state that pH influences the solubility of P, meaning a high pH will lead to precipitation, which makes nutrients unavailable for plants. The nutrient can precipitate as magnesium ammonium phosphate (struvite) (Eck et al., 2019). Siebielec et al. (2014) state that a pH above 7,0, can lead to much of the dissolved P reacting with calcium and forming calcium phosphates. The formation of insoluble compounds makes the PO_4^{3-} unavailable for plants. P is mainly present as $H_2PO_4^-$ in aquaponics, due to the pH range. The forms H_3PO_4 and HPO_4^{2-} occur at low levels. Plants absorb P as $H_2PO_4^-$ and HPO_4^2. When the pH increase, the uptake of PO_4^{3-} is lowered, due to a reduction in the $H_2PO_4^-$ content (Cerozi & Fitzsimmons, 2016). Whether precipitation or formation of unideal compounds occurred during the experiment is not known, but the literature shows that it may lead to unavailable forms of P, which can relate to the amount of P remaining in the water after a week. Water samples were not analyzed for the specific forms of P, other than PO_4^{3-}. For future aquaponics experiments, more extensive water analysis is recommended.

[1] PO_4-P is found by multiplying PO_4^{3-} by 0,3261 (0,8015x0,3261=0,26x100=26%) (HACH, 2022g)

Concentrations for other nutrients such as K, Ca, Mg, Cu and Zn were lower for GM1 and GM2 (Table 5), compared to recommended values in hydroponic solutions by Hoagland (Table 1). These results were expected as water from aquaculture is known to have a lower amount of nutrients, which complies with data from Bittsanszky et al. (2016). They saw that nutrient concentrations in aquaponics systems were significantly lower than in hydroponic systems, for most nutrients.

5.2.3 Plant Growth

The experiment shows that the plants grew during the week they were located in the system. The results on height and weight parameters indicate that plants in GM1 and GM2 developed similarly. The plants grew well, with an approximately 50% increase in height in a week, despite the low nutrient uptake. No difference was seen between plants grown in water from RAS and soil plants, thereby showing potential for growing plants in RAS. Total weight could not be measured for soil plants as they could not be removed from the soil. The growth media was therefore excluded from weight parameters. Plants could have been gently removed from the soil, roots washed, measured, and then replanted, but this process could affect the plants negatively.

After 7 days of the experiment, the plants had 6,58 (CP) to 7,39 (GM2) leaves. The results show similar leaf formation as found by Draghici et al. (2016), where 6 to 7,5 leaves were registered after 7 days in different organic nutrient solutions. GM1 and GM2 formed similar amounts of leaves, while GM1 had significantly more leaves than CP at the start, and GM2 had significantly more leaves than CP at the end of the experiment. This is contrary to findings by Lei and Engeseth (2021) who saw no significant difference in aerial lettuce growth in water (with hydroponic solution) and soil. This is not directly comparable, as no hydroponic nutrient was added to the RAS water used, but it also makes it extra interesting that hydroponic plants grew more than CP.

Combining leaf growth and visual registrations indicates that the hydroponic plants and soil plants developed differently. Visual registrations show small variations between soil and aquaponics plants, where the lettuce grown in water seems crispier and more compact, compared to the longer and more spread leaves seen in soil. This is contrary to findings by Ibrahim and Zuki (2012), saying no differences were found between soil and aquaponic plants

for sensory evaluations. Hess-Erga et al. (2013) experienced short and discolored leaves in lettuce grown in wastewater from RAS. This was not seen in the experiment, which can be related to the short amount of time the lettuce spent in the water (7 days).

Dry matter in hydroponic plants was between 5,08±0,29% to 5,15±0,62% which was significantly higher than in soil plants at 4,52±0,21%. No significant difference was seen between the two hydroponic parallels, indicating that the different water types did not significantly affect the concentration of DM. The percentage for hydroponic plants is slightly higher than what Anderson et al. (2017) found as dry matter in leafy greens, which was around 4%. Lei and Engeseth (2021) found lower DM contents in hydroponic plants compared to soil plants, which is the opposite of what is seen here. Still, this is not directly comparable as hydroponic plants were not grown in water from aquaculture. Madar et al. (2019) found in their research that DM accounted for 6,52±0,06% of FW in aquaponics, and similar numbers for plants in hydroponic solution. Results found in this research, therefore, seem to be within ranges seen in previous research.

Results on ash content were found to be an average of 20,48% of DM in GM1, 19,62% in GM2 and 23,97% in CP, and no significant differences were seen between the growth media. This relates well with Sularz et al. (2020) findings that ash content accounts for 18,35-22,00 g/100g of dry matter of full-grown lettuce (*Lactuca sativa cv. 'Melodion'*) grown in nutrient film technique. Ibrahim and Zuki (2012) found that lettuce (*Lactuca sativa cv. 'Grand Rapid'*) grown in soil had a higher AC than lettuce grown in hydroponics and aquaponics, which is also reflected in the results from this experiment.

5.3 Placement of Plant Production in RAS

The literature review and the experiment explore two water outtake points; after biofilter (GM1) and plate separator (GM2). In addition, wastewater is mentioned as a possible source. The three locations are marked in Figure 23.

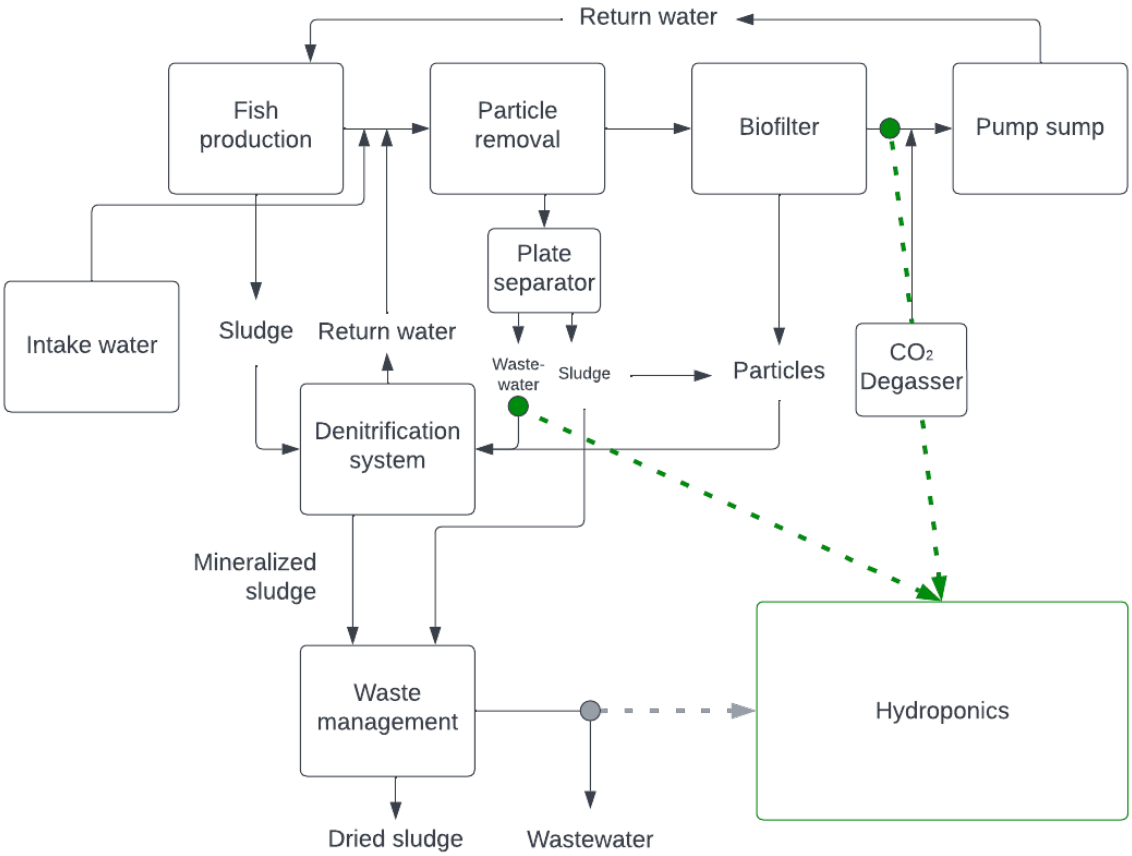

Figure 23 - A simplified and generalized overview of recirculating aquaculture systems based on AquaMaof and AkvaGroups systems. Green arrows show the points of water outtake in the experiment. The grey arrow indicates wastewater as a possible connection point.

The biofilter converts NH_3 to NO_3^-, indicating that the plant section should be placed after the biofilter in a RAS, for higher quantities of NO_3^- to be available (section 2.3.1). To see the actual effect of the biofilter, water for analysis was collected from a Norwegian smolt facility (anonymized) (Table 13). Samples of water before and after biofilter showed no changes in NO_3-N concentrations after nitrification. At a pH of 6,6 close to 0% of TAN will be NH_3 (section 2.3.1), and recommended value for aquaculture are to keep NH_3-N below 0,0125 mg/l (Appendix 2). This indicates that the nutrient concentrations are very low, and conversion of NH_3 to NO_3^- will not make much of a difference to the registered values of NO_3-N and NO_2-N.

The concentration may disappear in the selected decimal representation or measure uncertainties of 20%. Considering the uncertainty, NO_3-N levels can be within the range of 12000 to 18000 µg/l, which is very high compared to the NH_3-N level of 180 µg/l.

The topic was discussed with Kari Attramadal who is the Head of R&D at Nofitech and course coordinator for NTNU's RAS course. She says the NO_2-N and NO_3-N levels may be similar in the samples before and after the biofilter because the water moves quickly through the system, and the change in levels might be so small that it does not show due to the measurement uncertainty (Attramadal, K., Personal communication, February 2022). Attramadal states that whether you place the hydroponic part before or after the biofilter is not of significant importance, for this reason exactly. The most important factor is to place the plant section after mechanic filtration to lower the degree of particles in the water (Attramadal, K., Personal communication, February 2022).

According to the experiment NO_3-N and NO_2-N, NH_4-N and PO_4-P were removed with 5,4%, 94,8% and 33,8%, respectively (Table 5 – Results from the water analysis of water inputs and outputs of the experiment, performed by Eurofins. Values are given in mg/l and decrease in percent. Negative values in percent indicate increase in nutrient content, while minus alone represent missing data points.Table 5). Removal rates suggest that the placement can contribute to the filtering process of both N and P compounds, but that high levels of NO_3-N are not removed.

The other option explored is placing the hydroponics in combination with the early phases of sludge removal. Different RAS has different solutions for how to manage waste. Connecting the hydroponic section to the 'top water' from the plate separator will provide water with a similar concentration of dissolved nutrients as after the biofilter, except for higher amounts of NH_4-N (Table 5). The water consists of a higher degree of particles (Table 5), which can affect plant growth. Stout (2013) states that particles can stick onto roots and limit nutrient uptake. It would therefore be of high importance to include a settling tank or a mineralizer before the water enters the plant section. This relates well with information provided by Attramadal, on placing the plant section after mechanic filtration (Attramadal, K., Personal communication, February 2022). Goddek et al. (2018) and Delaide et al. (2019) see the mineralization of sludge through anaerobic mineralizers, as an important step to further utilize nutrients in the particles, stating that it may lower the need for the addition of commercial nutrients.

According to the experiment, NO_3-N and NO_2-N, and NH_4-N were removed by 32,35% and 99,58%, respectively, while PO_4-P increased by 12,86% (Table 5). Removal rates suggest that the placement can be a part of the N filtering process, but P compounds are not efficiently removed. Still, plants grew well in this growth media with regards to weight, height and leaf formation, but not significantly better than plants in water from the biofilter. Plants did not show any sign of deficiencies due to low nutrient uptake, which may be because of the short duration of the experiment. Plants being under such conditions for longer periods might show more severe deficiencies, as seen for leaf formation by Hess-Erga et al. (2013).

Both water from after biofilter and from the plate separator show potential to be a part of the filtration process, but removal rates are not high enough to replace other processes in RAS. Denitrification and de-phosphorus systems would still be needed to clean the water sufficiently. Still, the water from RAS is a resource that can be further used in a secondary growth section to utilize valuable nutrients and available water in a circular economy.

Wastewater is also of high interest for aquaponics but was not used in this thesis, as it was not possible to collect from Osan smolt facility at the time of the experiment (Øren, S. O., Personal communication, January 21, 2022). The wastewater has in some facilities been through processes of denitrification and de-phosphorus, meaning it has a lower nutrient content, and would not be as suitable for plant growth (AquaCon, 2022; AquaMaof, 2019). In Osan's facility 99% of total N and 24% of total P are planned removed before being emitted (Wæhre, 2017), showing a low potential for plant growth in this water, as plants optimally require high concentrations of N (220 mg/l NO_3^- and 12,6 mg/l NH_4^+), and P (24 mg/l) when grown hydroponically (Hoagland, 1950). Jakobsen et al. (2021) performed a similar experiment with the use of hydroponic solutions in combination with RAS wastewater (from a system without denitrification and de-phosphorus), and plants showed 30% higher growth with water from RAS in combination with hydroponic nutrients. Wastewater from systems without a zero-water exchange system contains more nutrients and can thus be more suitable for plant growth.

5.4 Aquaponics and Sustainability

Challenges with a growing global population, combined with the lack of freshwater resources and agricultural land, mean we must look towards new solutions for food production (FAO, 2017, 2018a, 2018b, 2019). Norway can contribute to the global food supply through aquaculture, and Sjømat Norge (2018) states a high increase in seafood production by 2050 is needed. As previously mentioned, production through RAS will allow for an increase in aquaculture, without the traditional challenges (Badiola et al., 2012), but will lead to a large amount of nutrient-rich waste. Goddek, Joyce, Kotzen, et al. (2019) see aquaponics as a solution for utilizing these nutrients and promoting a circular economy. In addition, they identify aquaponics as a farming approach that can have a positive effect on planetary boundaries with regards to freshwater use, land system change and biochemical flows. Thus, providing a flexible production method, which can help reach the SDG and develop more sustainable food systems. A similar conclusion was reached by Tyson et al. (2011), saying that aquaponics is close to sustainable agriculture as it combines the production of plants and animals, integrates nutrient flows by natural biological cycles such as nitrification, and has efficient use of valuable and nonrenewable resources. On the contrary, König et al. (2016) found a lack of data on large-scale commercial systems and found it difficult to conclude on the sustainability of the systems. Either way, integrating plant production in RAS can enable new areas for food production (Badiola et al., 2012), and sites not previously suitable can produce both aquatic species and vegetables (Baras, 2018). Both production methods can in combination consume less water and enable higher utilization of nutrients than more traditional production methods (Rakocy et al., 2006). The experiment performed show that plants grown in RAS water could produce as much biomass as soil plants.

6 Conclusion

Based on the results, this Master of Science project concludes that lettuce production (*Lactuca sativa)* can be used as part of the filtering process to remove N and P compounds from water in the RAS production of Atlantic salmon. The removal rates were found to be too low to replace denitrification and de-phosphorus processes, yet it can contribute to a small removal. NH_4^+ was efficiently removed from all hydroponic systems (>90%), while NO_3-N was removed in small amounts in GM1(<6%), and higher in GM2 (>30%). Parts of PO_4-P were removed in GM1 (>30%), while it increased in GM2 (+12,8%). Plants in water from RAS developed at similar rates as soil plants, regardless of the limited nutrient uptake. This shows a potential for plant growth in cold-water aquaponics and the possibilities for creating a more sustainable food production through further utilization of valuable nutrients.

The literature review combined with the plant experiment suggests that both after the biofilter and the top-water in the plate separator can be potential connection points for hydroponic plant sections. Leading a side stream of water into decoupled hydroponics systems where optimal conditions for both plant and fish can be secured, is found to be the most optimal solution for commercial production. Nutrient solutions to optimize plant growth can be added, as the RAS water does not contain sufficient levels of nutrients. By including a mineralization process more of the sludge can be utilized for plant growth, and the need for commercial nutrient solutions can be lowered.

7 Further Work

Further work would be to structure a larger and more advanced decoupled system directly linked to a side stream of a commercial system and include mineralization reactors to utilize solids as extra nutrients. Full analysis and continual registrations of physiochemical parameters and nutrients would give a better impression of the development and uptake over time. Performing a material flow analysis in a specific RAS with a connected hydroponics would be highly interesting, to see the actual nutrient levels throughout an aquaponics system. Few significant differences were observed during the experiment, which may be due to the experimental design. For future work adding more replicates can improve the experimental design and give more precise results.

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9 Appendix

9.1 Appendix 1

Nutrient for plant growth, chemical symbol, function in plant, and the necessary form for uptake by plants. Table based on information adapted from "Hydroponics Systems and Principles of Plant Nutrition: Essential Nutrients, Function, Deficiency, and Excess» by Sanchez (2020).

Nutrient	Chemical symbol	Function in plant	Form of nutrient for plant uptake
Macronutrients			
Nitrogen	N	Component of amino acids, nucleic acids, proteins, and coenzymes	NO_3^- and NH_4^+
Phosphorus	P	Membrane phospholipids, nucleic acids, ATP, NADP intermediates of metabolism	$H_2PO_4^-$ and HPO_4^{2-}
Potassium	K	Osmotic regulation, enzyme activation, and turgor	K^+
Calcium	Ca	Cell structure, signal transduction, and enzyme activation	Ca_2^+
Magnesium	Mg	Component of chlorophyll and enzyme activation	Mg_2^+
Sulfur	S	Component of sulfur amino acids, proteins, and coenzyme A	SO_4^-
Carbon	C	Components of organic compounds	CO_2 and H_2O
Hydrogen	H		
Oxygen	O		
Micronutrients			
Iron	Fe	Photosynthesis, respiration, and redox changes	Fe_2^+
Manganese	Mn	Essential for water splitting and enzyme activation	Mn_2^+
Copper	Cu	Photosynthesis, respiration, and redox changes	Cu_2^+
Zinc	Zn	Enzyme cofactor-activator	Zn_2^+
Boron	B	Cell division, membrane activity	BO_3^-
Molybdenum	Mo	Nitrate reduction, redox changes	$MoO4_2^-$

9.2 Appendix 2

Water Quality Criteria for Aquaculture of Salmon

Water Quality Criteria for Aquaculture	
Timmons and Ebeling (2010)	
Parameter	Concentration (mg/l)
Alkalinity (CaCO3)	50-300
pH	6.5-8.5
Dissolved Oxygen (DO)	<5
Carbon Dioxide (CO2)	<20
Total Dissolved Solids (TDS)	<400
Total Suspended Solids (TSS)	15
Ammonia (NH_3-N unionized)	<0,0125
Ammonia (TAN)	<1,0
Nitrite (NO_2^-)	<1 (0,1 in soft water)
Nitrate (NO_3^-)	0-150 and up
Phosphorus (P)	0,01-3,0
Potassium (K)	<5
Sulfate (SO4)	<50
Sulfur (S)	<1
Sodium (Na)	<75
Magnesium (Mg)	<15
Calcium (Ca)	4-160
Chloride (Cl)	<0,003
Copper (Cu)	
Alkalinity <100 mg/l	<0,006
Alkalinity >100 mg/l	>0,03
Iron (Fe)	<0,15
Manganese (Mn)	<0,01
Zinc (Zn)	<0,005

9.3 Appendix 3

Expert opinions from personal communication throughout the experiment

Name	Job position	Company	Opinion on	Date	Type of communication
Øyvind Mejdell Jakobsen	Senior researcher and Research manager, PhD	CIRiS	Plant nutrients	19.10.21	E-mail Meeting
Anonymous	Operations manager	Anonymous	RAS and waste products	Nov – feb 2022	Phone E-mail
Matthijs van den Boogaard	Water Quality Specialist	SalMar Settefisk AS	Aquaponics, RAS and waste products	05.10.21	E-mail
Svein Oluf Øren	Operations manager	Osan Settefisk	RAS and RAS layout	14.01.22 – 21.03.22	Online and personal meeting at facility
Øyvind Øksnes Dalheim	Leader R&D	Kvidul	RAS		Meetings
Damian Steinke	Land-based Aquaculture specialist	Kvidul	RAS		Meetings
Kari Attramadal	Head of R&D, Associate Professor II	Nofitech, NTNU	RAS, Aquaponics, waste products		Lectures and discussions between lectures
Olav Hilmarsen	Founder	Greenaquanor	Closed loop aquaponics	30.03.22	Online meeting

9.4 Appendix 4

Materials

Hydroponic system:

- 40l containers x 6 (Clas Ohlson)
- EPS (Bygger'n)
- Plant light (Nelson Garden, Led light)
- Airpump x 2 (BOYU air-pimp S-4000B)
- Airstones x 6 (Mikrogartneriet)
- Airhose x 10 m (Mikrogartneriet)
- Plastic pots x 12 (Ø12) (Mikrogartneriet)
- Soil (Premium plant soil) (Plantasjen)
- Netpots x 54 (Ø5) (Mikrogartneriet)
- Weight

Data collection from plants:

- Plants from experiment
- Weight
- Laboratory weight
- Folding rule
- 66 x Porcelain crucibles
- Dry oven
- Ash oven

9.5 Appendix 5

Water analysis performed with HACH probe (HQ40d multi). All chemical used were provided by HACH.

Materials:

- 14 beakers
- Mixer
- Deionized water
- Probe stand x 2
- Lint-free cloth
- HACH HQ40d multi (HACH, 2022a)
- HACH Intellical PHC101
    - pH buffer 4,0 and 7,0 (HACH, 2022e)
- HACH Intellical LDO101
    - BDO bottle, 300 ml (HACH, 2022d)
- HACH Intellical ISECL181
    - Chloride Ionic Strength Adjustor (ISA) Buffer Powder Pillows
    - Sodium Chloride (HACH, 2022b)
- HACH Intellical ISENH3181
    - Ammonia ISA powder pillow (1 pillow for 25ml of solution)
    - Ammonia Ionic Strength Adjustor (ISA) Solution
    - Ammonia Nitrogen Standard Solution, 100mg/l as NH3-N (25ml).
    - Nitrogen Ammonia Standard Solution, 1000-mg/L as NH3–N (HACH, 2022c)

Method:

pH and temperature (HACH Intellical PHC101) Electrode Method 8156. Probe was placed in beakers with 50 ml sample water, before the reading was performed (HACH, 2022e).

Dissolved oxygen (Intellical LDO101 LDO probe) Direct measurement method. Probe was placed in beakers with 50 ml sample water, before the reading was performed. Measurement range: 0,1- 20 mg/l O_2 (HACH, 2022d).

Chloride (Intellical ISECL181 combination chloride ISE probe)
Direct ISE method. 25 ml of sample water and a chloride ISA powder pillow was added per beaker. The solution was gently stirred before the probe was placed in the solution and the measurements were read. Measurement range: 3,55g/l to 35g/l Cl^- (HACH, 2022b).

Ammonia nitrogen (Intellical ISENH3181 ammonia ISE)
Direct ISE method. 25 ml of sample water was added in 50ml beakers, one powder pillow of ammonia ionic strength adjustor (ISA) was added per sample. The beakers were gently stirred to dissolve the ISA. Bubbles were removed from the probe by gently shaking it before the reading was performed. Measurement range: 0,01 mg/l to 14 000 mg/l. Accuracy of 0,05% (HACH, 2022c).

9.6 Appendix 6

Analyses performed by Eurofins, presented with analysis number, name, method and the reference method.

Analysis Number	Analysis	Method	Reference Method
MM164-2	pH at 23 +/-2°C	Potentiometry	NS-EN ISO 10523
MM166-5	Suspended Solids	Calculation	Intern Method
MM515-3	Total Phosphorus	Spectrophotometry (CFA)	NS-EN ISO 15681-2
MM519-3	Total Nitrogen	Spectrophotometry (CFA)	NS 4743
MM465-2	Nitrate + Nitrite ($\Sigma(NO_3+NO_2)$-N)	Spectrophotometry (DA)	NS-EN ISO 13395
MM463-1	Phosphate	Spectrophotometry (DA)	NS-EN ISO 15681-2
MM512-1	Ammonium (NH_4-N)	Spectrophotometry (DA)	NS-EN ISO 11732
MM0BE-1	Cu, filtered	ICP-MS	NS-EN ISO 17294-2:2016
MM0B9-1	Ca, filtered	ICP-MS	NS-EN ISO 17294-2:2016
MM0B8-1	K, filtered	ICP-MS	NS-EN ISO 17294-2:2016
MM0B6-1	Mg, filtered	ICP-MS	NS-EN ISO 17294-2:2016

9.7 Appendix 7

Water parameters measured during experiment.

1-A	Date	Air temperature	Water temperature	pH	Dissolved oxygen (mg/l)	EC (mS/cm)
Day 0	02.02.2022	23,3	21	7,7	8	4,46
Day 1	03.02.2022	22,9	23,1		8	
Day 2	04.02.2022	23,6	23,4	7,5	7,7	3,98
Day 5	07.02.2022	23,4	24,2	7,8	7,2	4,19
Day 6	08.02.2022	23,3	24,2		7,3	
Day 7	09.02.2022	22,9	23,7	7,96	7,7	4,42
1-B						
Day 0	02.02.2022	23,3	20,9	7,7	7,5	4,49
Day 1	03.02.2022	22,9	23,1		8	
Day 2	04.02.2022	23,6	23,5	7,8	7,3	4,01
Day 5	07.02.2022	23,4	24	7,8	7	4,21
Day 6	08.02.2022	23,3	24,3		7,3	
Day 7	09.02.2022	22,9	23,6	7,51	7,4	4,42
1-C						
Day 0	02.02.2022	23,3	21,1	7,85	7,4	4,49
Day 1	03.02.2022	22,9	23,3		8	
Day 2	04.02.2022	23,6	23,7	7,9	7,6	4,37
Day 5	07.02.2022	23,4	24	7,9	7,4	4,08
Day 6	08.02.2022	23,3	24,3		7,5	
Day 7	09.02.2022	22,9	23,6	7,94	6,8	4,43
GM1	**P-value[1]**		**0,989**	**0,161**	**0,483**	**0,854**

2-A	Date	Air temperature	Water temperature	pH	Dissolved oxygen (mg/l)	EC (mS/cm)
Day 0	02.02.2022	23,3	21	7,68	4	4,57
Day 1	03.02.2022	22,9	23,1		7,4	
Day 2	04.02.2022	23,6	23,4	7,9	7,7	4,49
Day 5	07.02.2022	23,4	23,9	7,8	6,9	4,34
Day 6	08.02.2022	23,3	24,4		7	
Day 7	09.02.2022	22,9	23,5	7,83	7,2	4,48
2-B						
Day 0	02.02.2022	23,3	21	7,66	5	4,59
Day 1	03.02.2022	22,9	23,2		7,3	
Day 2	04.02.2022	23,6	23,4	7,9	7,4	4,57
Day 5	07.02.2022	23,4	23,9	7,9	6,9	4,34
Day 6	08.02.2022	23,3	24,3		8,5	
Day 7	09.02.2022	22,9	23,6	7,89	7,6	4,49
GM2	**P-value[1]**		**0,981**	**0,657**	**0,580**	**0,724**
GM1/GM2	**P-value[2]**		**0,900**	**0,524**	**0,059**	**0,020**

[1] P-value to find differences between replicates within the growth (One-wayANOVA with replicate as factor). [2] P-value to find differences between growth media growth (One-wayANOVA with growth media as factor. Water temperature and oxygen: GM1 n= 18 (1-A n=6, 1-B n=6 and 1-C n=6), GM2 n=12 (2-A n=6 and 2-B n=6). EC and pH: GM1 n= 12 (1-A n=4, 1-B n=4 and 1-C n=4), GM2 n=8 (2-A n=4 and 2-B n=4).

9.8 Appendix 8

Pearson Correlation

Pearson correlation for physiochemical water parameters during experiment.

Water parameters		Number of samples	Correlation coefficient (r)	p-value	Correlation
pH	Water temperature	20	0,374	0,104	Medium correlation[2]
	EC	20	0,137	0,565	Weak correlation[1]
Water Temperature	EC	20	-0,171	0,470	Weak correlation[1]

[1]R<0,3 = weak correlation, [2]R>0,3<0,7 = medium correlation, [3]R>0,7 = strong correlation *p<0,05

Pearson correlation for physiochemical water parameters in water samples analyzed at lab.

Water parameters		Number of samples	Correlation coefficient (r)	p-value	Correlation
pH	Temperature	15	0,318	0,249	Medium correlation[2]
	Oxygen	15	0,486	0,066	Medium correlation[2]
	Ammonia	4	0,885	0,115	Weak correlation[1]
	Chloride	15	-0,052	0,854	No correlation
Ammonia	Temperature	4	0,694	0,306	Medium correlation[2]
	Chloride	4	0,968	0,032*	Strong correlation[3]
	Oxygen	4	0,939	0,061	Strong correlation[3]
Chloride	Temperature	15	0,004	0,988	Weak correlation[1]
	Oxygen	15	0,488	0,065	Medium correlation[2]
Oxygen	Temperature	15	0,004	0,988	Weak correlation[1]

[1]R<0,3 = weak correlation, [2]R>0,3<0,7 = medium correlation, [3]R>0,7 = strong correlation, *P<0,05

9.9 Appendix 9

Results from analysis of water parameter in water samples extracted during the experiment. Water input on day 0, mid of experiment on day 5 and water output on day 7.

Parameter and time of sample	GM1			P-value Within GM1[1]	GM2		P-value Within GM2[1]	P-value Between GM1 and GM2[2]
	1-A	1-B	1-C		2-A	2-B		
pH								
Average in GM		8,23±0,0		0,458	7,93±0,29		0,770	0,010
Water input	8,26	8,26	8,26		7,6	7,6		
Average in GM		8,26±0,0			7,6±0,0			
Water mid	8,34	8,08	8,15		8,32	7,96		
Average in GM		8,19±0,14			8,14±0,25			
Water output	8,25	8,39	8,1		7,98	8,1		
Average in GM		8,25±0,15			8,04±0,85			

Parameter and time of sample	GM1			P-value Within GM1[1]	GM2		P-value Within GM2[1]	P-value Between GM1 and GM2[2]
	1-A	1-B	1-C		2-A	2-B		
Temperature								
Average in GM		19,02±0,57		0,852	18,85±1,14		0,832	0,702
Water input	18,4	18,4	18,4		17,9	17,9		
Average in GM		18,4±0,0			17,9±0,0			
Water mid	19	19,1	19,2		18	18,5		
Average in GM		19,1±0,1			18,4±0,57			
Water output	19,7	20	19		20,3	20,2		
Average in GM		19,57±0,51			20,25±0,07			

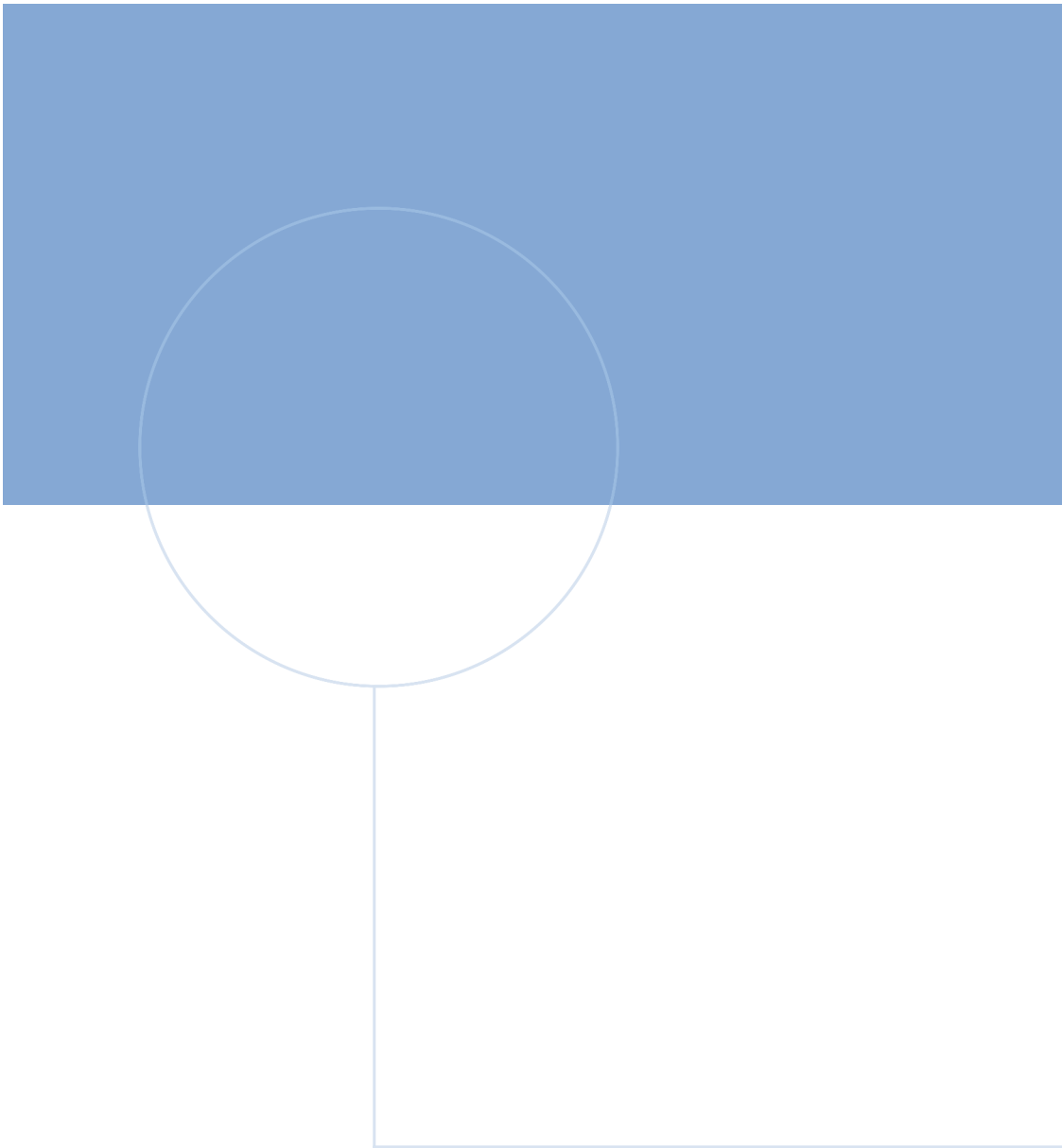
Parameter and time of sample	GM1			P-value Within GM1[1]	GM2		P-value Within GM2[1]	P-value Between GM1 and GM2[2]
	1-A	1-B	1-C		2-A	2-B		
Ammonia (NH_3-N)								
Average in GM		nd		nd	2,27±1,7		0,969	nd
Water input	<0,01mg/l	<0,01mg/l	<0,01mg/l		0,82	0,82		
Average in GM		<0,01mg/l			0,82±0,0			
Water mid	<0,01mg/l	<0,01mg/l	<0,01mg/l		3,63	3,81		
Average in GM		<0,01mg/l			3,72±0,13			
Water output	<0,01mg/l	<0,01mg/l	<0,01mg/l		<0,01mg/l	<0,01mg/l		
Average in GM		<0,01mg/l			<0,01mg/l			

Parameter and time of sample	GM1			P-value Within GM1[1]	GM2		P-value Within GM2[1]	P-value Between GM1 and GM2[2]
	1-A	1-B	1-C		2-A	2-B		
O_2 mg/l								
Average in GM		6,53±0,9		0,749	6,2±0,44		0,495	0,405
Water input	6,93	6,93	6,93		5,73	5,73		
Average in GM		6,93±0,0			5,73±0,0			
Water mid	6,77	6,59	7,37		6,82	6,42		
Average in GM		6,91±0,41			6,62±0,28			

Water output	5,51	7,14	4,63		6,46	6,01	
Average in GM		5,75±1,27			6,24±0,32		

Parameter and time of sample	GM1				GM2			
	1-A	1-B	1-C	P-value Within GM1[1]	2-A	2-B	P-value Within GM2[1]	P-value Between GM1 and GM2[2]
Cl mg/l								
Average in GM		934,4±110,9		0,964	927,5±104,9		0,534	0,905
Water input	1015	1015	1015		959	959		
Average in GM		1015±0,0			959±0,0			
Water mid	1021	991	966		1016	1001		
Average in GM		992,67±27,6			1008,5±10,6			
Water output	719	815	853		900	730		
Average in GM		795,67±69,7			815±120,2			

[1] P-value for differences between replicates within the growth media growth (One-wayANOVA with replicate as factor). [2] P-value calculated based on growth media growth (One-wayANOVA with growth media as factor). GM1 n=9 (1-A n=3, 1-B n=3 and 1-C n=3) and GM2 n=6 (2-A n=3 and 2-B n=3).



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