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The Growth and Development of Lettuce, Coriander and Swiss chard in a Cold Water Aquaponic System Optimized for Lettuce Production

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Master of Science in Plant Science

Preface

This master thesis was written at the Department of Plant Sciences at the Norwegian University of Life Sciences (NMBU). The experiments were conducted at the Norwegian Institute of Bioeconomics (NIBIO) in Landvik during the autumn and winter of 2015-2016. I am very happy to have been given the opportunity to write about a great passion of mine: The combination of fish and plant production in the same system. My choice of taking a Master's degree in plant science while simultaneously taking courses in fish farming and entrepreneurship reflects my interest in fish and plants. It is my dream to one day start my own business within this field. I hope that the food production of tomorrow becomes an environmentally friendly one, and that this thesis would contribute to increase the interest in urban food production in temperate and arctic climates.

I would like to thank my main supervisor Anne-Berit Wold for keeping an eye on the details and giving valuable advice during the writing of my thesis. I would also like to thank co-supervisors Randi Seljåsen and Siv Lene Gangenes Skar at NIBIO Landvik for your expertise and positivity during the experimental period of my thesis. A big thank you to Atle Beisland and other employees at NIBIO Landvik for making me feel at home while gathering data. I am grateful for the thorough feedback received from co-supervisor Anne Kjersti Uhlen and most of all for contacting NIBIO Landvik. This thesis would not have been possible without you. Thank you also to Bama and Gartnerhallen for your financial support, without which this thesis would have been much harder to accomplish.

And last but not least, thank you to my friends, to Lilja Elina Kaarina Palovaara Søberg and the rest of my family, for your motivation, input and everything else.

Ås, August 15th 2016

Erkki-Einar Søberg

Abstract

Aquaponic research seemed to be characterized by experiments conducted in warm water systems while experiments in cold water systems were almost non-existent per August 2016. The main aim of this thesis was therefore to produce more data on the growth and development of plant species and cultivars in a cold water system. One preliminary study and one comparative study was conducted to examine the growth and development of the lettuce cultivars ‘Frillice’ and ‘Salanova Excite R2’, the coriander cultivar ‘Marino’ and the swiss chard cultivar ‘Bulls Blood’ when grown in an aquaponic system optimized for lettuce production. No significant difference between any of the growth parameters (yield, fresh weight of leaves and roots, fresh weight of leaves per rockwool cube, height and number of leaves, length of roots) and production system was found ($p<0.05$). The nutrient concentrations of plants, fish and water were analyzed. The largest ‘Frillice’ yield of 19540 g obtained in the preliminary monoculture study was higher than reported from other cold water experiments, but lower than reports from warm water experiments.

Results from the comparative study showed that the highest aquaponic yield was produced by ‘Salanova Excite R2’ and ‘Frillice’. The average fresh weights of ‘Salanova Excite R2’ and ‘Frillice’ leaves were significantly higher than ‘Bulls Blood’ and ‘Marino’ leaves ($p<0.05$). The average leaf weights per rockwool cube of ‘Salanova Excite R2’ and ‘Frillice’ were significantly higher than the leaf weights per cube of ‘Marino’ and ‘Bulls Blood’ ($p<0.05$). ‘Marino’ produced both significantly heavier and longer roots when compared to the three other cultivars ($p<0.05$). ‘Salanova Excite R2’ obtained the highest number of leaves ($p<0.05$) and ‘Marino’ produced the tallest leaf average of all cultivars ($p<0.05$).

The average fish weights and lengths were 122.37 g, 22.07 cm, and 177.67 g, 23.41 cm for bleke (*Salmo salar L.*) and brown trout (*Salmo trutta*), respectively. Brown trout was recommended for cold water aquaponic production because it achieved a higher harvest weight and grew faster than bleke. Rainbow trout (*Oncorhynchus mykiss*) and freshwater prawns (*Macrobrachium rosenbergii*) might also be good options for nutrient production in aquaponic systems. Nutrient concentrations were similar in the preliminary and comparative studies with the exception of higher sodium concentrations being found in both aquaponic plants and water in the comparative study.

Sammendrag

Aquaponic forskning så ut til å være preget av eksperimenter utført i varmtvannsanlegg mens eksperimenter utført i kaldtvannsanlegg nesten ikke eksisterte per august 2016. Hovedmålet i denne oppgaven var derfor å produsere mer informasjon omhandlende vekst og utvikling av plantearter og sorter i et kaldtvannssystem. En innledende studie og en sammenliknende studie ble gjennomført for å undersøke vekst og utvikling av salatsortene 'Frillice' og 'Salanova Excite R2', koriandersorten 'Marino' og bladbetesorten 'Bulls Blood' i et akvaponisk system optimalisert for salatproduksjon. Det ble ikke funnet noen signifikant forskjell mellom vekstparameterne (avling, ferskvekt av blader og røtter, ferskvekt av blader per steinullkube, bladhøyde og -mengde, og rotlengde) og produksjonssystem ($p<0.05$). Næringsinnholdet i planter, fisk og vann ble analysert. Den høyeste 'Frillice' avlingen på 19540 g oppnådd i den innledende studien var høyere enn det andre kaldtvannseksperimenter oppnådde, men lavere enn det varmtvannseksperimenter oppnådde.

Resultatene fra den sammenliknende studien viste at 'Salanova Excite R2' og 'Frillice' produserte den høyeste akvaponiske avlingen. Gjennomsnittlig ferskvekt av 'Salanova Excite R2' og 'Frillice' blader var signifikant høyere enn 'Bulls Blood' og 'Marino' blader ($p<0.05$). Gjennomsnittlig bladvekt per steinulltkube for 'Salanova Excite R2' og 'Frillice' var signifikant høyere enn bladvekt per steinullkube for 'Marino' og 'Bulls Blood' ($p<0.05$). 'Marino' røtter var både signifikant tyngre og lengre sammenliknet med de tre andre sortene ($p<0.05$). 'Salanova Excite R2' produserte signifikant flere blader enn alle andre sorter ($p<0.05$) og den største gjennomsnittlige bladhøyden ble produsert av 'Marino' ($p<0.05$).

Gjennomsnittlig ferskvekt og lengde av fisk var 122.37 g, 22.07 cm og 177.67 g, 23.41 cm for bleke (*Salmo salar L.*) og brunørret (*Salmo trutta*). Brunørret ble anbefalt til kaldtvanns akvaponisk produksjon fordi den oppnådde høyere slaktevekt og vokste raskere enn bleke. Regnbueørret (*Oncorhynchus mykiss*) og ferskvannsreker (*Macrobrachium rosenbergii*) kan også være gode alternativer for produksjon av næringsstoffer i akvaponiske systemer. Næringsinnholdet var likt i de innledende og sammenliknende forsøkene med unntak av høyere natriumkonsentrasjoner funnet i både akvaponiske planter og vann i det sammenliknende forsøket.

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1. Introduction

Food production has traditionally been associated with friendly farmers living in rural landscapes. The increasing amount of people moving into urban city centers leaves the countryside with a decreasing population. This results in a subsequent increase in the resource needs of cities, resources such as food. Food is normally produced in the countryside and transported to stores in urban areas where consumers can pick and choose from a variety of options. The worldwide transportation of food is resource demanding and contributes to global warming. These and other reasons have led to increasing demands for locally produced, high quality food all across the western world (Det Kongelige Landbruks- og Matdepartement, 2011-2012, 19-21). The result of this is an increasing interest in both technology and food production systems that can be built within urban areas. Entrepreneurs have seen this shift in the market and invested in hydroponic (soilless) food production systems. This includes aquaponic systems that combine fish farming with hydroponic plant production (Diver, 2006; Rakocy, et al., 2006). Urban food production is gaining in popularity both as a hobby and as a commercial venture, especially in the U.S.A.

Climate change will affect the growing conditions of crops in many different ways, resulting in higher insecurities when it comes to achieving satisfactory yields in traditional agriculture. The increased environmental control offered by indoor food production might become a safer option in the near future. This could save crops that would otherwise fail due to environmental factors such as heavy rains or drought. Aquaponic food production provides an environmentally friendly alternative to hydroponic production. Aquaponic systems replace hydroponic fertilizers produced from non-renewable resources with nutrients from nutrient rich and organic fish wastes. Aquaponic systems produce crops of similar quality as hydroponic ones, with the advantage of producing fish as a byproduct (Rakocy et al., 2006). The fish yield is comparable to semi-intensive aquaculture (fish farms) while using less water (Al-Hafedh et al., 2008).

Research aiming to optimize this environmentally friendly option to future food production is increasing year by year, conferences are held and project collaborations are formed across multiple nations. Aquaponic food production may prove superior when compared to hydroponic production due to having free access to nutrients produced by fish that add to the bottom line (Rakocy et al., 2011). Most of the current research is based on warm water systems, while the literature offers very little in terms of cold water studies. Warm water aquaponic production may not be economically feasible in temperate and arctic climates as the increased heating costs may render year-round food production too expensive. It is important that research is conducted in both warm and cold water systems so that farmers of the future may use this research when deciding how to increase the urban food production of tomorrow.

This thesis consists of two studies that examine aquaponic food production in a cold water system located at NIBIO Landvik. The first study is part of a larger quantitative study that focuses on the biomass production of the lettuce (*Lactuca sativa*) cultivar 'Frillice', rainbow trout (*Oncorhyncus mykiss)* and brown trout (*Salmo trutta*). This first study serves as a preliminary experiment, producing data for later comparisons with the polyculture study of this thesis. The aim of the comparative polyculture study is to investigate the growth and development of the cultivars 'Salanova Excite R2', 'Frillice', 'Bulls Blood' and 'Marino' in a cold water system stocked with brown trout and bleke (*Salmo salar L.*). The following growth parameters will be used in the investigation: Yield, fresh weight of leaves and roots, fresh weight of leaves per cube, height and number of leaves, length of roots.

1.1. Objectives and research questions

The main objective of this study is to provide more data on the growth and development of the selected plant species in a cold water aquaponic system, thus contributing to the smaller pool of research papers investigating cold water aquaponics. The research questions of this thesis are as follows:

- What is aquaponic food production and how does it differ from hydroponic food production?
- How does that growth parameters of 'Frillice', 'Salanova Excite R2', 'Marino' and 'Bulls Blood' vary when the plants are grown in an aquaponic system compared to a hydroponic control?
- How is does the growth and development of these plant cultivars vary compared to literature and 'Frillice' in the preliminary and the comparative monocultures, when grown in a cold water aquaponic system optimized for lettuce production?
- How do water quality parameters affect the yields plant and fish crops?

2. Literature review

2.1. What is aquaponic food production?

Aquaponic food production is similar to hydroponic (soilless) plant production in many aspects. It combines the production of plants with fish farming in the same recirculating system (Rakocy et al., 2004b; Rakocy et al., 2006; Diver, 2006). The main difference between the two types of plant production is their nutrient source. While hydroponic systems use nutrient solutions based on liquid fertilizers as a nutrient source, aquaponic systems use waste water from aquaculture (fish farming). This waste water can be supplied just like the hydroponic nutrient solution, but is most often supplied by producing fish in the same system and recirculating the water between fish and plants. There are also minor operational differences. While both systems recirculate water, hydroponic systems need to renew their water more often than aquaponic systems. This is necessary due to changes in nutrient concentrations and pH as the plants absorb different amounts of many nutrients used for plant growth, resulting in nutrient toxicity if the water is not periodically dumped, renewed and mixed with a new nutrient solution. Aquaponic systems have a more stabile nutrient balance because nutrients are converted from fish excrements and uneaten fish feed continuously, giving the plants a continuous nutrient supply thus lowering fluctuations in nutrient concentrations. Hydroponic systems flood their plant roots with a highly concentrated nutrient solution that is diluted over time.

2.2. Development of hydroponic and aquaponic production systems – a historical overview

The discovery of hydroponic plant production came about when researchers Nicolas-Théodore de Saussure and Wilhelm Knop among others, were investigating which elements were essential to plant growth and development in the nineteenth century (Taiz & Zeiger, 2010). They grew plants in a soilless nutrient solution complete with inorganic salts, discovering nutrient deficiencies when they removed one element at a time. This demonstration also proved that plants are able to grow and develop normally on nothing but inorganic elements, water and sunlight (Taiz & Zeiger, 2010). Knop developed early nutrient solution formulations, while the modern modified Hoagland solution was developed by D. R. Hoagland. The modified Hoagland solution forms the basis of most nutrient solutions used today, and the majority of hydroponic farmers dilute this nutrient solution to suit their plant production (Taiz & Zeiger).

The origins of aquaponic food production originated in different parts of the world. Asian nations such as China and Thailand have been growing rice in fields flooded with nutrient rich water from fish ponds for centuries (Skar et al., 2015). Early aquaponic food production was one reason why the Aztecs in Central America were able to sustain their rapid population expansion, through food production on stationary islands called "Chinampas" (*Figure 2-1*). Chinampas were perfect for growing crops, consisting of nutrient rich mud, taken from the bottom of a lake and deposited in layers on top of wooden frames (Encyclopædia Britannica). This way of farming used nutrients from fish waste and decomposing plant material as an early fertilizer, making food production possible during a period in which the Aztecs had no access to land. The technique was so successful that it has been used for food production in Central America and other parts of the world up until this day. The contrast to the modernized, high-tech plant production factories of today is striking, but it all started with the same idea of reusing resources that were considered waste.

Figure 2-1: The Chinampas of the Aztecs (left) [1]. A modernized high-tech aquaponic system (right) [2].

Aquaponic production caught the interest of scientists at the University of Virgin Islands (U.S.A.), where a commercial scale system was constructed in 1994 (Rakocy et al., 1997). This aquaponic system became the model for most experimental designs for aquaponic research in climates ranging from tropical Israel (Kotzen & Appelbaum, 2010), Iran (Roosta & Hamidpour,

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[1] Retrieved from http://incredibleaquagarden.co.uk/-media/chinampa1.gif (2016, April 20).

[2] Retrieved from http://s.newsweek.com/sites/www.newsweek.com/files/styles/large/public/2014/05/12/5.16-urbanorganics02.jpg (2016, April 20).

2013) and Malaysia (Endut et al., 2009) to temperate Canada (Savidov et al., 2007) and Norway (Skar et al., 2015).

2.3. Hydroponic plant production systems and their suitability to aquaponics

The plant production part of aquaponic food production is often identical to hydroponic systems. These hydroponic systems can be divided into systems that allow constant water flow and systems that allow reciprocating water flow. The production systems that are most often used are raft, nutrient film technique (NFT) and ebb and flow systems (Figure 2-2). The raft, also called deep water culture (DWC) system, consists of plants that grow in plastic net pots, suspended in a floating raft with their roots standing directly in water. The plants grow in a pool of nutrient rich water that is constantly aerated. The raft system needs constant water flow and provides good growing conditions for small sized leafy vegetables and strawberries (Rakocy et al., 2006; Skar et al., 2015). Plants growing in the NFT system are placed in plastic net pots within holes cut into hydroponic trays or pipes. The water flows by gravitational forces due to a downward facing angle in the pipes. Water flows past the roots of the plants in a thin film, allowing the plant a continuous supply of water while simultaneously allowing the roots to breathe. NTF-systems should be run with a constant water flow. Running an NFT-system with

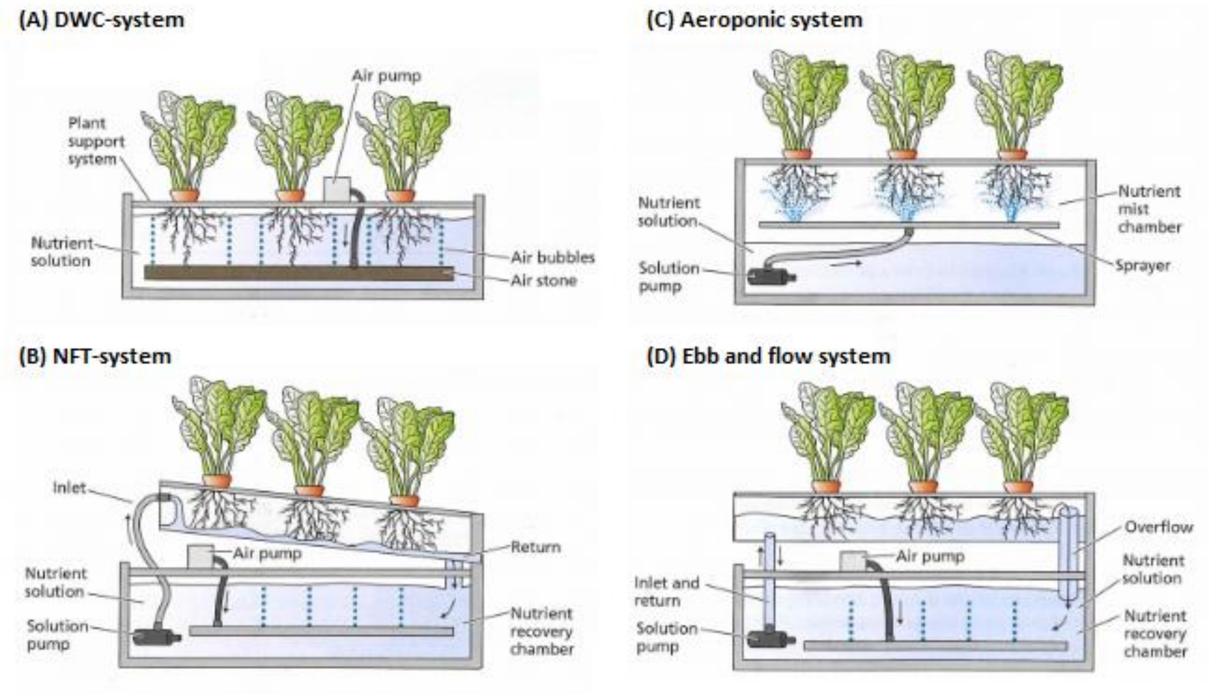


Figure 2-2: An illustration of four different hydroponic production systems. A Deep Water Culture (DWC) system in which plant roots grow submerged in oxygenated water (A), a Nutrient Film Technique (NFT) system in which plant roots grow in a thin water film (B), an Aeroponic system in which plant roots are suspended in air and sprayed with mist (C), and an ebb and flow system where the roots are flooded and drained periodically (D). Modified from (Taiz & Zeiger, 2010).

a reciprocating water flow reduced lettuce yields from 4.97 kg/m^2 to 4.34 kg/m^2 or 129.98 g/plant to 113.45 g/plant in an experiment conducted by Lennard & Leonard (2004). Plants that can be produced in a DWC-system can also be produced in a NFT-system.

The plants in an ebb and flow system grow in tanks filled with media such as gravel, sand or LECA-rock. The grow bed is regularly flooded with nutrient rich water that irrigates the plants, and the water drains out from the grow bed after a certain period of time, allowing air to reach the plant roots. This ebb and flow cycle is repeated indefinitely. Although mainly being a reciprocating system, it is also possible to turn the ebb and flow system into a constant flow system and adding oxygen, thereby making it a DWC-system filled with media. Smaller plants such as lettuce and herbs are well suited to ebb and flow-systems, but if the grow bed is deep enough it may support taller plants ranging from tomatoes to papaya trees (Rakocy et al., 2006; Hallam, M., N.D.). Aeroponic production systems are less known than the raft, NFT and ebb and flow systems. The plants growing in aeroponic systems are placed in plastic net pots with their roots hanging down into a chamber that is filled with nutrient rich mist (Taiz & Zeiger, 2010). This mist is sprayed directly onto the roots through pipes and a pump situated in a pool of nutrient solution at the bottom of the chamber. NFT, DWC, and aeroponic systems can be used to grow the same plants, of which, leafy vegetables are the most common.

DWC systems are the most preferred hydroponic system for research oriented facilities (Al-Hafedh et al., 2008; Bathia & Wasiim, 2012; Pantanella et al., 2010; Petrea et al., 2013b; Rakocy et al., 2004a; Rakocy et al., 2006; Rakocy et al., 2011; Sace & Fitzsimmons, 2013; Savidov, et al., 2007; Skar, 2015; Tyson et al., 2011; Vermeulen & Kamstra, 2013). NFT-systems are also popular, but not to the same extent as DWC, while the ebb and flow-system is more popular amongst small-scale, hobby sized aquaponic systems. Research done by Lennard & Leonard (2006) showed significant differences ($p<0.05$, $n=60$) between the yields of Green Oak Lettuce (*Lactuca sativa*) produced on waste from Murray Cod (*Maccullochella peelii peelii*) depending on production system. Lettuce yields were 5.05 kg/m^2, 4.47 kg/m^2 and 4.13 g/m^2 for an ebb and flow system filled with gravel media, DWC and NFT, respectively. The fresh weights per plant were 131.97 g, 116.91 g and 107.95 g, for ebb and flow, DWC and NFT systems, respectively. The ebb and flow system filled with gravel with continuous water flow produced the highest yield of lettuce while NFT produced the lowest (Lennard & Leonard, 2006).

There are different advantages and disadvantages with each system that limit their suitability for plant production. Plants grow quicker in the aeroponic system than in the other three, mainly because the roots have access to air and get nutrients sprayed directly onto them (Taiz & Zeiger, 2010). The roots have to extract nutrients from the whole water volume in the other hydroponic systems which leads to a slower growth rate. The disadvantage of aeroponic plant production is the higher risk of wilting and crop loss if a power outage occurs. The roots will dry out very quickly if the nutrient rich water spray stops. The same problem may cause a complete crop loss in NFT systems as well, as the plant roots growing in NFT systems are partially covered by a thin water film. Ebb and flow systems are somewhat safer depending on the drain cycle. If the system is designed with a slow fill and a quick drain, or a quick fill and slow drain the media still contains some moisture during the power outage. The DWC system is by far the most safe, as the roots always stay submerged. Pump failure will affect the oxygen content of the system water, but there will not be any risk of wilting.

Another problem with aquaponic plant production is clogging due to build-up of organic solids. Aeroponic and NFT-systems have a higher risk of crop failure due to sprays and pipes clogging, resulting in water blockages and subsequent drought damage (Rakocy et al., 2006). The clogged pipes may also lead to anaerobic zones within the systems disrupting the flow of oxygen to the roots, changing the nutrient balance and other parameters in the root zone of the plants. Clogging and formation of anaerobic zones may also happen in ebb and flow systems if the filter systems are sub-optimal. DWC systems are less prone to these problems because the grow beds are free of media and contain only water while oxygen is added through air pumps or as liquid oxygen. Good cleaning practices and well dimensioned filter systems minimize these risks due to greatly reduced amounts of organic build-up.

Two strategies that are widely used in hydroponic and aquaponic plant production are staggered and batch production. Staggered production is continuous plant production in which a portion of the total yield is harvested at regular intervals such as once weekly (Rakocy et al., 2006). The newly harvested plants are replaced by seedlings or younger plants, ensuring a balance between harvested and replaced plants allowing continuous production throughout the year. Staggered production is well suited for plants with low production times such as leafy greens and herbs. Fish can also grow in a staggered production, where fingerlings (young fish) are introduced multiple times throughout the growing cycle, resulting in fish of different age groups growing in separate tanks in the same aquaponic system. One age group of fish are harvested when it reaches marketable size and is replaced by new fingerlings allowing continuous production of fish as well. Batch production is mostly used for seasonal crops that have long production times. Plants are transferred into the systems as seedlings and allowed to mature before being harvested all at once, emptying the system before the next batch of plants is introduced. Tomatoes and other fruiting crops can be produced in this way, where the plants are removed once fruit production sub-optimal (Rakocy et al., 2006). Most aquaponic systems choose a staggered production in order to ensure a healthy balance between nutrient production by fish and bacteria, and nutrient removal by plants.

2.4. The challenge of finding an optimal nutrient balance in aquaponic systems

The balance between nutrient input and nutrient uptake is a key element to the success of aquaponic systems. If the amount of fish is increased without increasing the amount of plants, this leads to an increase in nutrient production while the nutrient removal stays the same. This will result in a buildup of ammonia, nitrite and other minerals ultimately leading to fish mortalities, shutting down all nutrient production. The reason behind the increased nitrogen concentrations is that *Nitrosomonas* sp. and *Nitrobacter* sp. are not able to increase their population numbers enough to convert the excess ammonia into nitrites and nitrates (Tyson, 2007). Other nutrients will also accumulate. Nutrient deficiencies will develop quickly if the amount of plants are increased without increasing the number of fish due to insufficient nutrient production. Nutrient deficiencies often lead to low quality plants that are harder to sell.

Fish feed is the main nutrient source for plants grown in aquaponic systems. Uneaten fish feed and fish waste that would be regarded as contaminants and toxins in traditional aquaculture, are transformed into high quality, liquid plant fertilizer by bacterial activity. The nutrients enter the aquaponic system water as fish feed. Fish respiration and break down of fish feed and feces produce highly toxic ammonia. 10 % of the protein content in the fish feed is transformed into ammonia (NH_3) nitrogen that then dissolves into ammonium (NH_4^+) in water following this equation (Taiz & Zeiger, 2010):

$$NH_3 + H_20 \rightarrow NH_4^+ + OH^-$$

Ammonia concentration is second only to oxygen concentration in importance when it comes to water quality factors affecting fish health (Tyson et al., 2011). Ammonia is toxic to both plants and animals because high concentrations will reduce the activity of photosynthetic and respiratory electron transport. High body concentrations of nitrate, although less toxic than ammonium, can lead to a condition called methemoglobinemia in which nitrate is reduced to nitrite that inhibits the ability of hemoglobin to bind oxygen (Taiz & Zeiger, 2010). Traditional recirculating aquaculture facilities remove excess toxins from their system water mechanically and biologically at great costs. Aquaponic systems share this waste treatment, but the costs are reduced because the biological filters operate at a higher efficiency (Rakocy et al., 2006). This is due to better conditions for biological nitrification, a process in which ammonia oxidizing bacteria of the genus *Nitrosomonas* sp. transform ammonia into nitrite (NO_2^-) while *Nitrobacter* sp. transform nitrite into nitrate (NO_3^-). DWC systems provide plenty of surface area for nitrifying bacteria underneath rafts and on all surfaces within the plant tanks. This means that the aquacultural biofilters can be replaced or reduced because of plant tanks in aquaponic systems supplementing these biofilters. The optimal temperature and pH ranges for maximum nitrification rates are at temperatures of 25–30 °C and a pH range of 7.0–9.0. Plants remove nitrogen both as ammonium and nitrate (Taiz & Zeiger, 2010). While ammonium usually is transformed into amino acids right after assimilation, nitrate has to be reduced to nitrite and then into ammonium before being transformed into amino acids. Uptake of both ammonium and nitrate is beneficial for plant growth because the two nitrogen forms help maintain a healthy cation-anion balance within plant tissues. Nitrogen is one of the most important nutrients for plant growth. An overview of the nitrogen cycle in aquaponics is shown in Figure 2-3.

The balance between nutrient production and nutrient removal can be achieved by using an optimal ratio of hydroponic plant growth area to fish growth area. It can also be achieved by feeding an optimal amount of fish feed per square meter daily. Table 2-1 shows that there is no clear consensus on the optimal ratios in literature. The ratios presented range from 0.5 – 7.3, and seem to depend on the type of aquaponic system and water temperature. The 0.5 was recommended in an ebb and flow system filled with gravel, whereas the same author has proven great success in the University of Virgin Islands (UVI) system operating with a 7.3 ratio. The optimal fish feed amount was based on DWC systems. A ratio of 25 % of the values recommended for DWC systems was recommended for ebb and flow and NFT-systems due to higher nutrient concentrations around the plant roots (Rakocy et al., 2006). The results from Iceland and Norway showed satisfactory, but nutrient deficient growth of lettuce and mizuna at the values shown in Table 2-1, while results from Denmark did not mention crop quality (Skar et al., 2015). Al-Hafedh et al. (2008) found that a ratio of 1.9 produced the highest romaine yield although ratios ranging from 1.2 – 7.5 was examined.

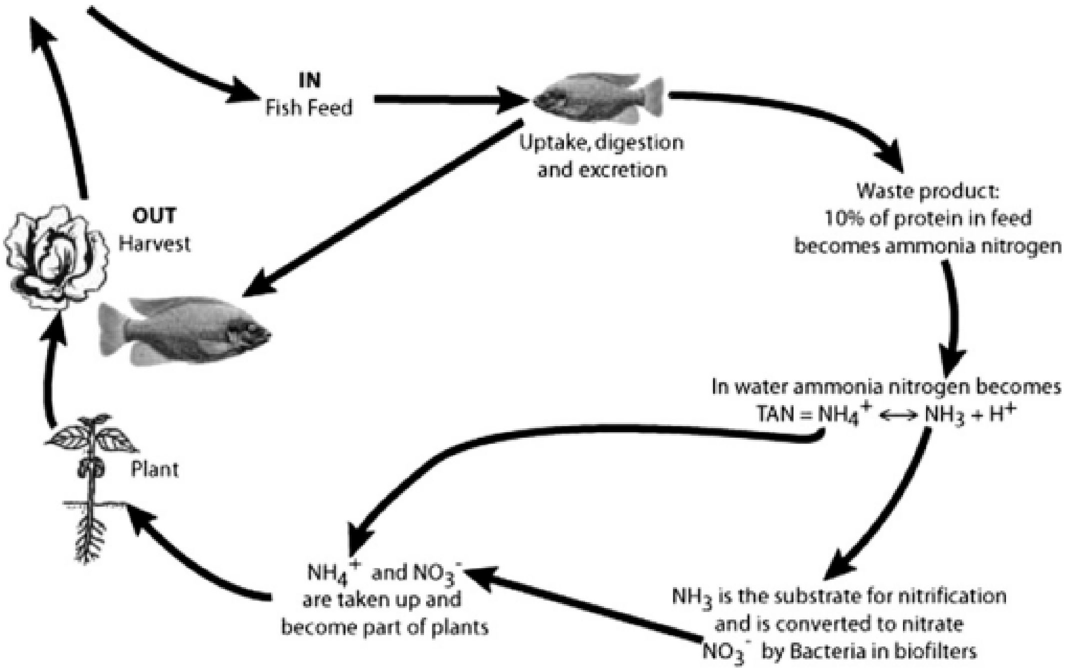


Figure 2-3: The nitrogen cycle of aquaponic system water. Nitrogen enters fish as fish feed and leaves fish as feces and urine, is transformed into ammonia nitrogen which is transformed into nitrite and nitrate through bacterial nitrification (Tyson et al., 2011).

Table 2-1: Different approaches to optimal plant growth area : fish area and fish feed rates to achieve nutrient balance within an aquaponic system.

Parameter	Al-Hafedh et al., 2008	Rakocy et al., 2006	Rakocy et al., 2004a	Skar et al., 2015 Iceland	Skar et al., 2015 Norway	Skar et al., 2015 Denmark
Plant area m^2 : fish area ratio m^2	1.2 – 7.5	0.5	7.3	2.5	2.0	-
Fish feed (g/day/m^2)	56	60-100	99.6	100	36.4	48

Aquaponic nutrient solutions are often poorer than hydroponic ones which sometimes lead to nutrient deficiencies render whole crops unsalable. Some nutrient deficiencies can however be negated by foliar application of a suspected deficient nutrient (Roosta & Hamidpour, 2013). Foliar application of potassium (K), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) increased the nutrient content of tomato leaves grown in an aquaponic system, but there was no change in nutrient content of tomato fruits. "[N]utrient concentrations will increase, decrease, or remain constant over time if nutrient production by fish is greater than, less than, or equal to nutrient assimilation by plants and nutrient losses, respectively" (Seawright, et al., 1997). Seawright et al. (1997) also claims that optimal nutrient concentrations only can be maintained through continuous monitoring and supplementation of elements that cause deficiencies. This summarizes the challenge in keeping nutrient concentrations within levels that lead to optimal plant growth conditions. The nutrient content of the aquaponic water depends on the nutritional content of the fish feed. Seawright et al. (1997) suggests that it is theoretically possible to construct fish feed that satisfy both the nutritional requirements of fish and plants without nutrient build up. Finding this optimal feed content would reduce or completely remove the need for nutrient supplements in aquaponic food production. The study of Seawright et al. (1997) showed that it is possible to manipulate the nutrient concentrations of K, Mg, Mn, phosphorous (P), sodium (Na) and Zn through fish feed composition, while Fe and Cu concentrations remained unchanged. Nutrient accumulation may also become problematic and even toxic. A total dissolved solids (TDS) concentration of above 2,000 ppm or 3.5 mmho/cm in electrical conductivity (EC) leads to phytotoxic (inhibitory or toxic to plants) conditions (Rakocy et al., 2006). Research done by Sace & Fitzsimmons (2013) shows that Chinese cabbage requires a TDS level of 1750-2100 ppm for optimal growth. This shows that plant species have different needs and tolerances of TDS levels. Zn can reach concentrations four to sixteen times higher in aquaponic systems than hydroponic systems, which can lead to Zn poisoning in fish.

Fish feed are designed to fulfill the nutritional needs of fish, resulting in low concentrations of elements that are not needed for fish growth. This leads to a discrepancy between the nutrient input through fish feed and the nutrient requirements of fish and plants.

Table 2-2 shows the composition of macro- and micronutrients of two commercial fish feeds. The measured nutrient content varies from one type of feed to the other. This may be due to Seawright et al. (1997) using feed for cat fish, while Rafiee & Saad (2004) were using feed for either tilapia or pangasius. The fish feed shown in

Table 2-2 contain higher amounts of nitrogen (N), P, K, calcium (Ca), Mg and Na than Fe, Zn and Cu. This looks very similar to the higher nutrient concentrations of macronutrients shown in Table 2-3 and lower micronutrient concentrations. The tables cannot be compared directly because the fish feed is given as % of dry feed while the nutrient solution concentrations are given in ppm. It does however show that increasing the nutrient percentage of dry feed could potentially match the nutrient concentrations in hydroponic plant solutions, unless the increased nutrient content would become toxic to fish.

Table 2-2: The composition of two commercial, floating fish feeds. Seawright et al. (1997) used a catfish diet (Rangen, Buhl, ID). The values are modified from Seawright et al. (1997). Rafiee & Saad (2004) used fish feed from Car-gill Company.

	Macro- and micronutrients in % of dry feed									
Source	N	K	Ca	Mg	P	Fe	Mn	Na	Zn	Cu
Seawright et al., 1997	-	1.28	1.61	0.30	1.48	0.0544	0.0161	0.47	0.0384	0.0018
Rafiee & Saad, 2004	3.40	0.53	1.74	0.428	1.48	0.1094	0.003	-	0.0056	0.0024

Table 2-3: The nutrient concentration of different hydroponic nutrient solutions. Jacks' Hydro-FeED used for lettuce production, Jack's Hydroponic used for herbs while the Modified Sonneveld's solution is used for leafy greens. The University of Arizona Controlled Environment Agriculture Center (UA CEAC) Recipe is used for tomato, cucumber and peppers (Mattson & Peters, N.D.). The Modified Hoagland solution is used for similar plants, while dilutions can be used for lettuce production (Taiz & Zeiger, 2010). Nutrient concentrations are in parts per million (ppm).

Nutrients	Jack's Hydro-FeED (16-4-17)	Jack's Hydroponic (5-12-26) + Calcium nitrate	Modified Sonneveld's solution	UA CEAC Recipe	Modified Hoagland solution
N	150	150	150	189	224
P	16	39	31	39	62
K	132	162	210	341	235
Ca	38	139	90	170	160
Mg	14	47	24	48	24
Fe	2.1	2.3	1.0	2.0	1.00-3.00
Mn	0.47	0.38	0.25	0.55	0.11
Zn	0.49	0.11	0.13	0.33	0.13
B	0.21	0.38	0.16	0.28	0.27
Cu	0.131	0.113	0.023	0.05	0.03
Mo	0.075	0.075	0.024	0.05	0.05

Table 2-3 shows that Jack's HydroFeED, Jack's Hydroponic, and the modified Sonneveld's solution can all be used to grow lettuce, swiss chard and coriander, because these plants are all leafy greens or herbs. The conventional fertilizer recommendations for field grown lettuce is 16 (12-20) N, 4 (2-6) P, 14 (12-16) K per kg/acre, while field grown beet recommendations are 14 (12-16) N, 4 (3-4) P, 16 (15-18) K per kg/acre (Yara a). Both of these are close to Jack's Hydro-FeED (16-4-17) recipe, but a direct comparison is unwise because the recommendations differ between field and hydroponic production.

2.5. Factors affecting growth and development of plants

The definition of plant growth is “an irreversible increase in volume” (Taiz & Zeiger, 2010). Classical plant growth analytics has focused on measuring the size (mass) or cell number of plants, but growth can also be measured by changes in fresh weight or dry weight. Growth curves can be used to describe the change in size, weight or dry weight over a certain time period. Plant growth depends both on genetic and environmental factors (Bævre & Gislerød, 1999). Cultivars of the same species can look completely different and produce vastly different yields. Environmental factors affecting plant growth are CO2, light and day length, temperature, relative humidity, pH and nutrient availability all of which interact and affect each other.

It is possible to achieve a higher degree of control of these environmental factors when producing plants inside a greenhouse where manipulation of growth factors is a requirement of modern plant production (Kimball and Idso, 1983; Bazzaz & Sombroek, 1996). If the light intensity inside a greenhouse increase, the temperature will also increase leading to increased CO_2 demand by the plants. The end result will be lower CO_2 levels and an increase in plant growth. Adding CO_2 in greenhouses will result in higher or lower growth rates, yields, water use and biological nitrogen fixation depending on plant species. Greenhouses with aquaponic systems will have increased CO_2 concentrations compared to hydroponic greenhouses without CO_2 enrichment because of fish respiration. Adding CO_2 to is a common practice in greenhouse production of lettuce and fruiting vegetables because it may increase yields by up to 30 % (Becker & Kläring, 2015). Aquaponic food production might reduce the need of CO_2 addition while still producing similar lettuce yields, thus increasing the economic viability of greenhouse production.

Optimal light levels are vital for plant growth as it together with CO_2 and water provide the building blocks of photosynthesis from which plants get all their energy (Taiz & Zeiger, 2010). The maximum photosynthetic assimilation differs between sun plants that adapted to open-field light conditions, and shade plants that adapted to living underneath other plants. These plant types have evolved different light harvesting mechanisms that suit their habitats. Shade plants can be damaged by light stress if they receive light intensities of 180-250 $\mu mol/m^2/s$ that are well suited to sun plants. Sun plants may react with reduced growth if they are grown in shade plant light intensities (Bævre & Gislerød, 1999; Taiz & Zeiger, 2010). The day length also influences growth and development rates of certain plants, especially if they are day length sensitive. Short day plants require longer periods of dark while long day plants require shorter periods of dark for inducing flowering.

Changes in temperature will affect plants in many different ways. Plant respiration, biomass increase, development phases as well as reproductive processes are all closely linked to temperature and temperature changes (Bazzaz & Sombroek, 1996). Cucumber (*Cucumis* sativus) plants produce more flowers in lower temperatures when compared to higher temperatures. The short day plant poinsettia delays flowering when grown in a higher nighttime temperature than daytime temperature, known as negative difference (DIF) (Myster & Moe, 1995).

Petunia plants respond with longer elongation when grown in higher daytime temperatures than nighttime temperatures, and respond with shorter elongation when grown in lower daytime temperatures than night time temperatures (Kaczperski et al., 1991). They found that the difference was larger at a lower light intensity, suggesting that both temperature and light intensity affect plant growth. The temperature of the root zone affects the uptake of water, nutrients and the development of the roots. The uptake rate of P and Fe decreases in lower root zone temperatures (Taiz & Zeiger, 2010). Higher temperatures lead to higher growth rates up to an optimum temperature, as the activity of all biological processes increase with increasing temperatures. The relative humidity affects the vapor pressure gradient between the air outside and inside the leaves. Low relative humidity leads to a large pressure gradient that increases transpirational water loss and vice versa.

A majority of scientific literature discusses aquaponic plant production in warm water systems (Al-Hafedh et al., 2008; Blidariu et al., 2013; Graber & Junge, 2009; Lennard & Leonard, 2006; Palm et al., 2014; Pantanella et al., 2010; Rakocy et al., 2004a; Rakocy et al., 2004b; Sace & Fitzsimmons, 2013; Savidov et al., 2007; Seawright et al., 1997; Sikawa & Yakupitiyage, 2010; Skar et al., 2015; Tyson, 2007). Papers discussing cold water systems are few in comparison (Buzby et al., 2016; Petrea et al., 2013a; Petrea et al., 2013b; Sace & Fitzsimmons, 2013; Skar et al., 2015;) per August 2016. The reason for this might be that most aquaponic papers originate from warmer climates. Aquaponic production in temperate or arctic climates depend other success factors than tropical climates. One example is that the production facilities must be protected from low winter temperatures and placed in heated greenhouses or other structures. The increased costs associated with aquaponic production in temperate regions due to heating and supplemental lighting may prove the single most important limitation for research on cold water systems. Scientific papers from U.S.A (Buzby et al., 2016), Iceland, Norway and Denmark (Skar et al., 2015) are vital in adding new knowledge on the performance and capabilities of cold water aquaponic systems in a scientific community dominated by warm water research. Economic reasons or an impression that aquaponic production only worked in warmer climates may be to blame for the low number of cold water research. Researchers in temperate and arctic climates have recently become more interested in cold water systems as an alternative to heating greenhouses to temperatures that allow tilapia production. This interest might result in a future hotspot for aquaponic research in arctic or temperate climates as opposed to the aquaponic facility at the University of Virgin Islands (UVI) that is located in a tropical climate.

There are many differences between a warm water and cold water aquaponic system, of which the most obvious is temperature. Both air and root zone temperatures influence growth, but the latter has greater effects on growth and nutrient absorption (Taiz & Zeiger, 2010). Growth rate of most plants and fish slow down as the temperature decreases. The same is true for the nitrifying bacteria, resulting in a slower nitrification rate in cold water aquaponic systems. The relative oxygen concentration of water increases as the temperature decreases (Dalsgaard et al., 2012), which benefit plants with lower tolerance of anaerobic root conditions. "Sensitivity analysis indicated that a temperature increment at 20 °C resulted in [a] nitrification rate increase of 1.108% per °C and 4.275% per °C under DO and TAN limited conditions, respectively" (Zhu & Chen, 2002). The maximum specific growth rate of nitrifying bacteria was determined to be a "monotonically increasing function of temperature in the range of 15–25°C" (Antoniou et al., 1990). This means that the rate of nitrification increases with the specific growth rate of nitrifying bacteria when the water temperature increases. Cold water systems with temperatures close to the lower end of the temperature range determined by Antoniou et al. (1990) would therefore naturally have a slower bacterial growth rate and a subsequent lower nitrification rate. These lower temperatures are below the optimal temperature range of 25–30 °C to achieve maximum nitrification rates (Rakocy et al., 2006). The temperature also limits which fish and plant species are able to grow in a cold water system.

The most used production practices are growing different species in the same system (polyculture) and growing one species in the same system (monoculture). Both have been practiced throughout our agricultural history. Aquaponic food production combines plant production with fish, providing an excellent example of successful use of polyculture. Monoculture is dominating in developed countries due to a desire to maximize the yield of one specific crop. Cereal and maize fields are examples of crops that have been bred to grow well with in the higher competition for light and nutrients observed within monoculture crops. The planting densities of traditional agricultural crops are cultivar dependent and follow plant spacing recommendations that promote optimal yields.

The phytocrome system senses changes in the red:far red (R:FR) ratio of light (Taiz & Zeiger, 2010). Shading decreases the R:FR ratio promoting shade avoidance responses resulting in lower leaf area and branching and increased internode elongation. This is a plant response that is very prominent in monoculture crops due to breeding for uniform growth and environmental factors such as light competition. A study by Petrea et al. (2013a) found that the maximum height of spinach varied between 14.46 cm at a plant density of 59 plants/m^2, 17.28 cm at a plant density of 48 plants/m^2 and 28.97 cm in a plant density of 39 plants/m^2 ($p<0.05$). Corresponding fresh weights of 2.78 g/plant in a plant density of 59 plants/m^2 was found to be significantly lower than 3.88 g/plant in a plant density of 39 plants/m^2 ($p<0.05$). The final leaf area varied significantly between 253.08 cm^2 in a plant density of 59 plants/m^2, 438.51 cm^2 at 48 plants/m^2 and 569.15 cm^2 at 39 plants/m^2 ($p<0.05$). Their experiments showed that the plant size, yield and leaf area ratio were all lower at higher plant densities than at lower plant densities.

A healthy nutrient balance is essential for successful food production regardless of production system. The nutritional needs of plants are different depending on their developmental stage. Germinating seedlings get their nutrients from the seed, while seedlings assimilate nutrients from their surroundings. Vegetative and generative growth also requires different levels of nutrients (Taiz & Zeiger, 2010). Recirculation of a hydroponic nutrient solution eventually leads to unbalanced nutrient concentrations due to many factors, including the fact that plants have a stronger affinity for certain nutrients than others and that an increase in pH leads to precipitation of minerals. An example of this is iron which is added in chelated forms such as sodium ferric diethylenetriaminepentaacetate (NaFeDTPA) in order to minimize precipitation in alkaline conditions thus keeping it available to plants. Na can become toxic to plants if the concentration gets too high in the presence of chloride (Cl) (Rakocy et al., 2006). Na concentrations higher than 50 mg/L will interfere with the plant uptake of K and Ca and may lead to higher concentrations of Na and nutrient deficiencies of K and Ca within plant tissues. Increased K concentrations will affect the uptake of Mg and Ca while each of the two other nutrients will have the same effect on K uptake when they are in excess (Rakocy et al., 2006).

Plants are dependent on essential elements in order to complete their life cycles. An essential element is, according to Arnon & Stout (1939) and Epstein & Bloom (2005), defined as “one that is an intrinsic component in the structure or metabolism of a plant or whose absence causes severe abnormalities in plant growth, development, or reproduction”. Essential elements are usually classified as macronutrients or micronutrients, based on their concentration within plant tissues. Table 2-4 shows the composition of essential elements and the dilution of a modified Hoagland nutrient solution traditionally used in hydroponic plant production. Hydrogen (H), carbon (C) and oxygen (O) are not included because these essential elements are obtained from water or carbon dioxide.

Table 2-4: The composition of a modified Hoagland nutrient solution for growing plants. Nickel is normally present as a contaminant added with other chemicals, so it may not be needed. Silicon should be added first to prevent precipitation of the other nutrients, followed by pH adjustement with HCl. NaFeDTPA = sodium ferric diethylenetriaminepentaacetate (Taiz & Zeiger, 2010).

Compound	Molecular weight	Concentration of stock solution	Concentration of stock solution	Volume of stock solution per liter of final solution	Element	Final concentration of element	
	g/mol	mM	g/L	mL		µM	ppm
Macronutrients							
KNO_3	101.10	1,000	101.10	6.0	N	16,000	224
$Ca(NO_3)_2$ * $4H_20$	236.16	1,000	236.16	4.0	K	6,000	235
$NH_4H_2PO_4$	115.08	1,000	115.08	2.0	Ca	4,000	160
$MgSO_4$ * $7H_2O$	246.48	1,000	246.49	1.0	P	2,000	62
					S	1,000	32
					Mg	1,000	24
Micronutrients					Cl	50	1.77
KCl	74.55	25	1.864	2.0	B	25	0.27
H_3BO_3	61.83	12.5	0.773		Mn	2.0	0.11
$MnSO_4$ * H_2O	169.01	1.0	0.169		Zn	2.0	0.13
$ZnSO_4$ * 7 H_2O	287.54	1.0	0.288		Cu	0.5	0.03
$CuSO_4$ * $5H_2O$	249.68	0.25	0.062		Mo	0.5	0.05
H_2MoO_4 (85% MoO_3)	161.97	0.25	0.040				
NaFeDTPA	468.20	64	30.0	0.3-1.0	Fe	16.1-53.7	1.00-3.00
Optional							
$NiSO_4$ * $6H_2O$	262.86	0.25	0.066	2.0	Ni	0.5	0.03
Na_2SiO_3 * 9 H_2O	284.20	1,000	284.20	1.0	Si	1,000	28

Hydroponic nutrient solutions contain varying amounts of essential elements. An unbalanced nutrient solution may lead to nutrient deficiencies that disrupt plant metabolism and function if the concentration of some essential elements is too low (Taiz & Zeiger, 2010). Nutrient deficiencies are not always easy to diagnose, as deficiencies of several elements may contribute to visible symptoms. Deficiencies of one element and excessive accumulations of another may happen due to different affinities for different essential elements. Nutrient deficiencies can also occur without producing any visible symptoms. There are also pests and diseases, especially viruses that can result in disease symptoms that resemble nutrient deficiencies.

Crop quality is perhaps the most important factor in commercial plant production because consumers demand healthy and fresh looking food. Pests and diseases are a bigger cause of concern in aquaponic systems than in hydroponic systems (Rakocy et al., 2006; Blidariu & Grozea, 2011). Very few pesticides and fungicides are legal to use on greenhouse crops. Hydroponic systems can be sprayed with minimal consequences, while aquaponic systems cannot. No legal pesticides can be used in a plant production system with fish because they can harm or kill the fish. Therapeutants and medicines to fight fish pests and diseases can be absorbed by plants and cause harm to crops or recipients of crops, and are therefore prohibited. The main strategy used to reduce the chance of potential crop loss due to pests and diseases is to prevent pest and disease establishment by using hardy plants and fish that are resistant or tolerant of them. Biological agents such as ladybugs or parasitic wasps can be used to reduce disease and pest pressure once the crop is under attack. The plant seeds should not be treated with chemicals to reduce the risk of fungicides and insecticides being released into the system water and harming or killing the fish that supply the nutrients for the plants. Nutrient deficiencies resulting in leaf discoloration can be just as bad as damage from pests or diseases because they can render whole crops unsalable. Fertilizing is done both to prevent nutrient deficiencies and increase yields. Avoiding nutrient dificiencies is the main reason why hydroponic growers replace their nutrient solutions regularly. Aquaponic growers do not need to replace their nutrient solutions, but the nutrient balance must still be maintained within healthy limits.

2.6. Plant and fish selection in aquaponic systems

Commercial plant and fish producers have different criteria for crop selection than researchers. Factors like market value, production time and temperature/light requirements are important to producers while researchers may expand on these factors and add crops regardless of their commercial value. The main criteria are never the less dependent on water quality parameters such as temperature, nutrient requirements and tolerance, dissolved oxygen concentration and pH. Production of warm water fish in cold climates is possible, but the cost of heating water often makes it economically unfeasible. The minimum germination temperatures of lettuce, coriander and swiss chard is 15 °C, 16 °C and 8 °C, respectively (LOG seed catalogue). Parsley (*Petroselinum crispum*) and the lettuce (*Lactuca sativa*) cultivar 'Crispy' produced good yields in an aquaponic system operating at 14 °C (Skar et al., 2015). Many plant species and cultivars including cilantro and swiss chard cultivars were produced in 13 °C (Buzby et al., 2016). Spinach (*Spinacia oleracea*), lettuce, chinese cabbage (*Brassica rapa pekinensis*) and pac choi (*Brassica rapa*) have been grown in water temperatures from 16.2-24.0 °C (Petrea et al., 2013a; Petrea et al., 2013b; Sace & Fitzsimmons, 2013). The cold water systems found in literature range in temperature from 13 °C (Buzby et al., 2016), 16 °C (Skar, et al., 2015; Sace & Fitzsimmons, 2013) to 17.8 °C (Petrea et al., 2013a; Petrea et al., 2013b).

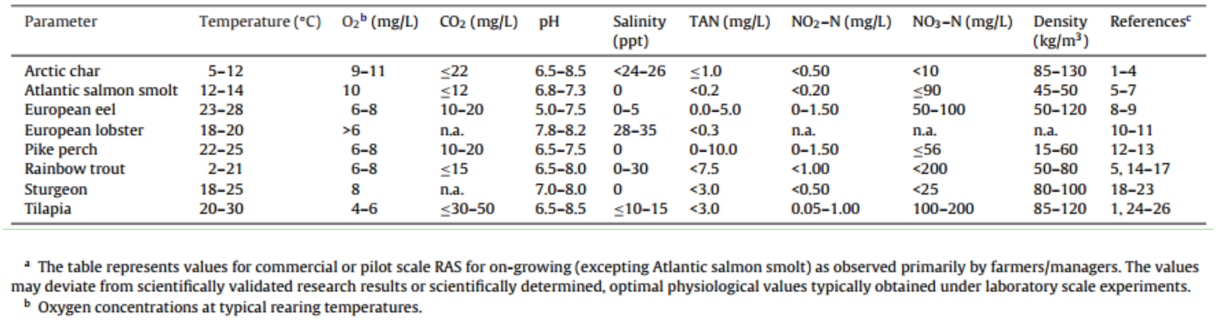
Tomato and cucumber plants that have an optimal growth temperature in the range of 25 – 32 °C are not suited for the lower temperatures found in cold water systems and will show sub-optimal growth (Palm et al., 2014; Savidov et al., 2007). Evidence of root crops such as carrots or potatoes were not found in scientific literature, although hobbyists have seen some success when experimenting with them. Production of root vegetables are only possible in ebb and flow systems either with continuous or reciprocating water flows filled with media because DWC and NFT systems are not designed for root crop production. Aeroponic systems could potentially succeed in producing root crops such as potatoes. The design of the hydroponic plant tanks in aquaponic systems therefore limit the optimal plant production to mostly produce leafy vegetables or fruiting vegetables.

The research conducted at UVI and other facilities in warmer climates may explain the observed higher number of fish, plant species and cultivars tested in warm water systems. Lettuce is the most studied plant species in the reviewed literature (Al-Hafedh et al., 2008; Blidariu et al., 2013; Buzby et al., 2016; Lennard & Leonard, 2006; Palm et al., 2014; Pantanella et al., 2010; Rakocy et al., 2004b; Sace & Fitzsimmons, 2013; Seawright et al., 1997; Sikawa

& Yakupitiyage, 2010; Skar et al., 2015). Spinach was the second most researched plant species (Bathia & Wasiim, 2012; Palm et al. 2014; Petrea et al., 2013a; Petrea et al., 2013b; Savidov et al., 2007; Skar et al., 2015). Tilapia was the most studied fish species (Al-Hafedh et al., 2008; Graber & Junge, 2009; Palm et al., 2014; Pantanella et al., 2010; Rakocy et al., 2004a; Rakocy et al., 2004b; Rakocy et al., 2011; Sace & Fitzsimmons, 2013; Savidov et al., 2007; Seawright et al., 1997; Skar et al., 2015; Tyson, 2007).

The same selection criteria that is used as a basis for the choice of plant crop(s) determine if a fish species is suitable for cold water aquaponic production. Arctic char, rainbow trout, trout and European lobsters will thrive in the temperature range found in most cold water aquaponic systems, while tilapia and European eel need warmer temperatures (Table 2-5). The choice of fish also depends other the water quality parameters of the aquaponic system. Stocking fish that tolerate the aquaponic water quality increases animal welfare and the maximum yields of the selected fish. This is the reason why a healthy nutrient balance is vital for the successful production of fish. Nutrient concentrations can be kept within the tolerance limits of most fish species when bacteria and plants assimilate efficient amounts of toxic ammonia, nitrite and nitrate. Fish that are bred specifically for aquaculture purposes such as rainbow trout produce higher yields than less domesticated fish. It is also possible to replace fish with other aquatic organisms such as fresh water prawns (*Macrobrachium rosenbergii*) (Sace & Fitzsimmons, 2013), although they are much less studied than fish. Table 0-1 in Appendix B presents an overview of different fish and plant species that are studied in aquaponic literature. The table includes information on the water temperature and pH of the aquaponic systems in which the fish have been produced. This table should be used as a tool when choosing fish and plant species for an aquaponic system. The purpose of the table is to provide a quick way to find out which fish and plant species have been studied at which temperatures, and to guide the reader to relevant literature thus making the crop choice easier.

Table 2-5: A generalized overview of the levels of water quality parameters tolerated by aquatic organisms in commercial or pilot recirculating aquaculture systems (RAS), from Dalsgaard et al. (2012) and references therein.



Parameter	Temperature (°C)	O$_2$[b] (mg/L)	CO$_2$ (mg/L)	pH	Salinity (ppt)	TAN (mg/L)	NO$_2$–N (mg/L)	NO$_3$–N (mg/L)	Density (kg/m^3)	References[c]
Arctic char	5–12	9–11	≤22	6.5–8.5	<24–26	≤1.0	<0.50	<10	85–130	1–4
Atlantic salmon smolt	12–14	10	≤12	6.8–7.3	0	<0.2	<0.20	≤90	45–50	5–7
European eel	23–28	6–8	10–20	5.0–7.5	0–5	0.0–5.0	0–1.50	50–100	50–120	8–9
European lobster	18–20	>6	n.a.	7.8–8.2	28–35	<0.3	n.a.	n.a.	n.a.	10–11
Pike perch	22–25	6–8	10–20	6.5–7.5	0	0–10.0	0–1.50	≤56	15–60	12–13
Rainbow trout	2–21	6–8	≤15	6.5–8.0	0–30	<7.5	<1.00	<200	50–80	5, 14–17
Sturgeon	18–25	8	n.a.	7.0–8.0	0	<3.0	<0.50	<25	80–100	18–23
Tilapia	20–30	4–6	≤30–50	6.5–8.5	≤10–15	<3.0	0.05–1.00	100–200	85–120	1, 24–26

[a] The table represents values for commercial or pilot scale RAS for on-growing (excepting Atlantic salmon smolt) as observed primarily by farmers/managers. The values may deviate from scientifically validated research results or scientifically determined, optimal physiological values typically obtained under laboratory scale experiments.

[b] Oxygen concentrations at typical rearing temperatures.

2.7. Yields of lettuce, coriander and swiss chard

The yields of lettuce, coriander and swiss chard obtained in aquaponic systems vary greatly from one source to another probably due to differences in cultivars and growing conditions (Table 2-6). Savidov et al. (2007) obtained a yearly lettuce yield of 28 kg m^{-2}. The individual fresh weight of lettuce varied between ca. 6 g – 327 g depending on source, cultivar and treatment. An aquaponic system with a growth medium consisting of sand produced the highest lettuce yield while the DWC control produced the lowest ($p<0.05$) (Sikawa & Yakupitiyage, 2010). The literature does not always specify if the lettuce yield includes the roots or if it is the total or marketable yield. The variation in lettuce yields can be explained by differences between cultivars. Romaine cultivars form heads thus increasing their fresh weight compared to red and green leaf lettuce that do not form heads.

The yields of coriander seem to be harder to determine from literature, as *Coriandrum sativum* L. is known by different English names depending on the origin of the English language used in scientific papers. The plant is called coriander and its seeds are called coriander seeds in the United Kingdoms, while the same plant is called cilantro and its seeds are called coriander in the U.S.A. (2016, July 27. Retrieved from https://delishably.com/spices-seasonings/coriander-cilantro, on 05.07.2016). This leads to confusion when reviewing available literature as to what parts of the plant are harvested because not all papers mention the specific plant partsin their materials and methods.

The yields of cilantro and coriander were 8 kg m^{-2} $year^{-1}$ and 12 kg m^{-2} $year^{-1}$, respectively (Savidov et al., 2007). The coriander fresh weight ranged from 0.2 g – 35 g/plant depending on treatment and source, in which a low water flow treatment produced the highest fresh weight (Buzby et al., 2016). One reason behind the lowest fresh weight was a fungal infection of *Rhizoctonia* or *Pythium* (Silva et al., 2015). It is important to note that coriander roots, plants and seeds are used as spice in Asian cuisines, which means that the roots may also be considered salable produce and contribute to the total coriander yield (Verma, A. et al., 2011).

A swiss chard (*Beta vulgaris* ssp. *vulgaris*) yield of 51.5 kg m^{-2} $year^{-1}$ was found by (Savidov et al., 2007) while Buzby (2016) reported a ‘Bulls Blood’ fresh weight of between ca. 0.7 – 2 g/plant, primarily depending on nutrient availability. Other swiss chard cultivar fresh weights varied between ca. 1.20 – 1.5 g/plant and ca. 1.6 –3.5 g/plant (Buzby et al., 2016). Swiss chard and beets are harvested for both their leaves and their roots (Freidig & Goldman, 2014). Majara

(2014) noted that 'Bulls Blood' is produced as a microgreen and babyleaf plant to compliment commercial salad mixes, resulting in early harvests of smaller leaves and/or roots.

Table 2-6: Yields of lettuce, coriander and swiss chard produced in aquaponic systems, gathered from literature. The numbers in parenthesis indicate what reference the data was found in. LF = Low water flow, HF = High water flow. FW = Fresh weight. N.D. = No data.

Plant crop	Cultivar	Yield	References
Lettuce	N.D.	28 kg m^{-2} $year^{-1}$	Savidov et al., 2007
	'Simpson'	173.73 g FW m^{-2}, Styrofoam (1) 423.40 g FW m^{-2} sand (1) 271.13 g FW m^{-2} gravel (1) 73.1–78.5 g/plant FW (2)	Sikawa & Yakupitiyage, 2010 (1); Sace & Fitzsimmons, 2013 (2)
	'Integral'	2.37–2.71 kg m^{-2} 5.67–5.70 kg m^{-2} 118.6–135.3 g/plant FW 283.3–285.2 g/plant FW	Pantanella et al, 2010
	Romaine 'Parris Island'	327 g/plant FW	Rakocy et al., 2011
	Romaine 'Jericho'	314 g/plant FW	--------||--------
	Red leaf 'Sierra'	269 g/plant FW	--------||--------
	Green leaf 'Nevada'	265 g/plant FW	--------||--------
	Bibb 'Rex'	LF: ca. 21 g/plant FW HF: ca. 45 g/plant FW Amended: ca. 35 g/plant FW	Buzby et al., 2016
	Butterhead 'Rhazes'	LF: ca. 6 g/plant FW HF: ca. 6 g/plant FW Amended: ca. 17 g/plant FW	--------||--------
Coriander		0.2 g/plant FW (1) 0.23 g/plant root FW (1) Cilantro 8 kg m^{-2} $year^{-1}$ (2), coriander 12 kg m^{-2} $year^{-1}$ (2) LF: ca. 35 g/plant FW (3) HF: ca. 30 g/plant FW (3) Amended: ca. 28 g/plant FW(3)	Silva et al., 2015 (1); Savidov et al., 2007 (2); Buzby et al., 2016 (3)
Swiss chard	N.D.	51.5 kg m^{-2} $year^{-1}$	Savidov et al., 2007
	'Bulls Blood'	LF: ca. 1 g/plant FW HF: ca. 0.7 g/plant FW Amended: ca. 2 g/plant FW	Buzby et al., 2016
Swiss chard	N. D.	LF: ca. 1.5 g/plant FW HF: ca. 1.25 g/plant FW Amended: ca. 1.20 g/plant FW	--------||--------
	'Early Wonder T. T'	LF: ca. 2 g/plant FW HF: ca. 1.6 g/plant FW Amended: ca. 3.5 g/plant FW	--------||--------

2.8. The future potential of aquaponics

Food that earlier was produced in fields has been transferred into greenhouses and buildings while the growing media has changed from soil to soilless production in hydroponic and aquaponic systems. Hydroponic plant production has a much lower water consumption compared to field grown plants that only absorb 10 % of the irrigation water given to them. Aquaponic systems save even more water as the aquaponic water does not need to be replaced at regular intervals. There is an increasing trend in which the general population craves ecological, chemical free food. Aquaponic plant and fish production is able to provide exactly this, as both fish and plants are produced in an ecological way without any chemicals.

International regulations are expected to reduce the negative environmental effects of aquaculture, especially when it comes to wastewater dumping (Blidariu & Grozea, 2011). This could place limitations on the fish production of flow-through and recirculating aquaculture facilities, even though fish farming is the fastest growing food sector in the world. Hydroponic farmers and aquaculture producers are already converting to aquaponic systems which supports the notion that aquaponic systems might provide both the salad ingredients and the meat of tomorrow (Savidov et al., 2007). Challenges in achieving an optimal nutrient balance between the production and assimilation of nutrients within the aquaponic system, controlling pests with biological agents and a greater variety of both plant and fish crops should be researched further to pave the way for this environmentally friendly food production system. Cold water aquaponic systems could make the whole year production of plant crops in temperate and arctic climates possible without increasing water temperatures to suit warm water crops. This would make aquaponics more economically viable, especially when aquaponic systems are able to produce similar yields to hydroponic systems while simultaneously producing fish as a byproduct.

3. Materials and methods

Two different experiments were conducted from August 2015 to January 2016. The first preliminary experiment studied the growth and development of the lettuce (*Lactuca sativa*) cultivar ‘Frillice’ in a monoculture. The second experiment compared the growth and development of four different plant species and cultivars. These were the lettuce cultivars ‘Frillice’ and ‘Salanova Excite R2’, the coriander (*Coriandrum sativum* L.) cultivar ‘Marino’ and swiss chard (*Beta vulgaris* ssp. *vulgaris*) cultivar ‘Bulls Blood’. The experiments were carried out in a cold water aquaponic facility located at NIBIO Landvik (58.34º N, 8.52º E, 58° 20′ 27.4″N, 8° 31′ 24.6″E. Supplemental lighting kept the photoperiod at 16h light and 8h dark when natural sunlight provided too little light. The greenhouse containing the aquaponic system occupied 150 m^2 and was situated approximately 10 meters above sea level.

3.1. The system design of the aquaponic facility at NIBIO Landvik, Norway

The research facility at Landvik produced the lettuce cultivar ‘Frillice’ in a staggered culture, harvesting plants once a week. Rainbow trout (*Oncorhyncus mykiss)* and brown trout (*Salmo trutta*) were producing nutrients for the plants. Rainbow trout was later replaced by a freshwater salmon (*Salmo salar L.*) locally known as bleke (Torgersen and Terofal, 1980). The aquaponic system was a 100 % recirculating DWC-system that had been in continuous operation for more than a year prior to the start of the studies conducted within this thesis. The total water volume of the aquaponic system was 10 m^3, consisting of two plant growth beds (6 m^3), four fish tanks (2,4m^3), four swirl separators, aeration tank, sump, bead- and biofilters and piping. The fish tanks were covered with shade cloth to reduce light levels by 86 % thus decreasing fish stress and algal growth in open tanks. The two plant grow beds have a combined growing area of 20 m^2. The aquaponic system had a planting density of 33,6 plants per m^2, while the hydroponic control had a planting density of 36 plants per m^2. The hydroponic control and the aquaponic germination chamber were the same size and volume, situated close to the main aquaponic system. This facility is able to produce 416 kg of lettuce and 360 kg of fish per year (Skar et al., 2015). Figure 3-1 shows a technical drawing of the aquaponic system and its components at Landvik.

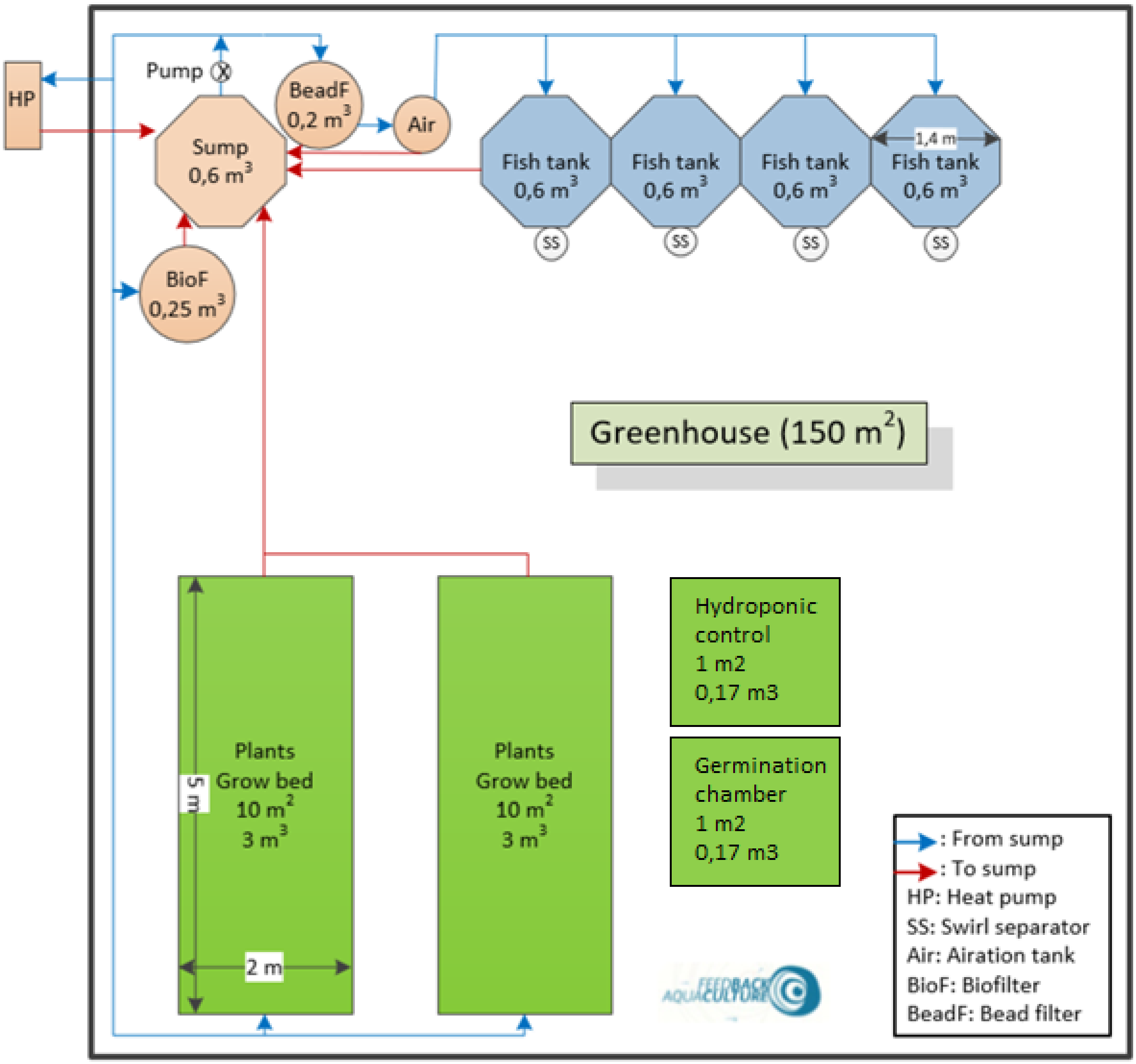
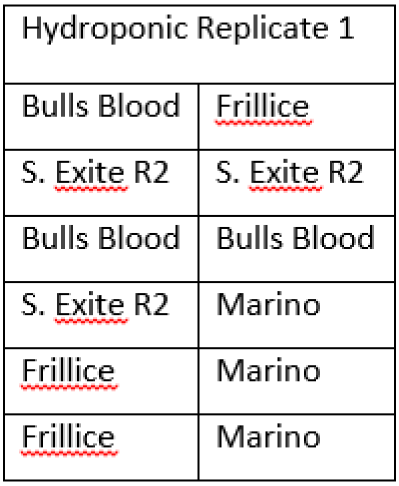


Figure 3-1: A technical drawing made by AqVisor AS showing the set-up of the aquaponic facility including the hydroponic control and aquaponic germination chamber at NIBIO Landvik, modified from Skar et al. (2015). Explanations for the figure are found at the bottom right.

The experimental set-up of the preliminary study of 'Frillice' in monoculture followed an already established production plan and used the whole aquaponic production system. The comparative study was confined to four plant trays in the southernmost part of the left plant grow bed in Figure 3-1. The comparative study consisted of four plant trays, each being treated as a replicate, complete with all four plant species and cultivars. The location of the cultivars was completely randomized within each tray. While Figure 3-2 shows replicate 1 of the experimental set up from the hydroponic control, the complete experimental set-up of both the hydroponic and the comparative study can be found in Appendix A.



Hydroponic Replicate 1	
Bulls Blood	Frillice
S. Exite R2	S. Exite R2
Bulls Blood	Bulls Blood
S. Exite R2	Marino
Frillice	Marino
Frillice	Marino

Figure 3-2: The experimental set-up for the hydroponic control (left), and part of a table describing the plants within replicate number 1 (the two plant rows at the far left) of the hydroponic control. The complete experimental set-up can be found in Appendix A.

Table 3-1: Technical information about the fish and plant production capacity of the aquaponic facility during 2015-2016. Modified from Skar et al. (2015). shows an overview of the technical information concerning the aquaponic production system, including the amount of fish feed being the main nutrient supply added to the system on a daily basis, the ratio of plant growth to fish tanks area and other parameters. The table is divided into ratios and water treatments, where the latter explains what technology is used to clean the system water as well as how the system parameters are monitored. The moving bed biofilter is filled with K1 Kaldnes media while the Polygeyser DF-6 beadfilter is filled with enhanced nitrification bead media. The system water flows in one of two paths. It is either pumped up from the sump and into the fish tanks, flowing through the swirl separators and into some of the filters before returning to the sump, or pumped up from the sump and flowing into the hydroponic plant grow beds before returning to the sump. The system is pumping water from the sump and letting the water flow back into the sump with gravitational forces (Figure 3-1).

Table 3-1: Technical information about the fish and plant production capacity of the aquaponic facility during 2015-2016. Modified from Skar et al. (2015).

Parameter	Unit
Ratios	
Recirculation ratio	100 %
g fish feed/day per m^2	36,4
Fish : plant ratio	1:4.8
Water treatments	
Swirl separators	Each fish tank, 17 l each
Particle filter, m^3	Bead filter, 0.4 m^3
Sludge water handling	Aerob stabilization
pH-control	$CaCO_3$, $K_2CO_3^-$
Biofilter, m^3	Moving bed, 0.25 m^3
Aeration/oxygenation/CO_2-stripping	Down-flow aeratiors, in-tank aeration
UV-irradiation	No
Sedimentation, m^3	No
Temperature control	Heat pump, aquarium heater (hydroponic c.)
Other supplements	Chloride, Iron
In-line measurements	pH, temp, O_2, water flow

Water quality parameters were monitored both automatically and by manual samplings. Computer systems monitored water quality parameters by processing information from sensors. Water and air temperature, relative humidity (RH), dissolved oxygen (DO), pH, $CaCO_3$ and electrical conductivity (EC) were all monitored. The data was logged by an analog AAC 3100 data logger. Water temperature and oxygen content was measured by a Sentronic SentrOxy WQM-sensor. Air temperature and RH was measured by a temperature sensor from Biltema AS (Art. 84-0860). pH was measured by a Hamilton Polilyte Plus 120 pH-sensor, through a Knick Pikos signal converter and an M-System M2XU Universal Transmitter before values were sent to the data logger. The natural decline in pH was prevented by automatically adding lime slurry made up of $CaCO_3$ and water. A Watson-Marlow 313 peristaltic pump was used to dose the slurry when necessary. pH was later adjusted manually by adding $K_2CO_3^-$. The computer system also included an INTAB Tinytag mA single channel logger complete with display and a PR electronics 2289 signal converter that displayed water quality parameters on a screen (Skar et al., 2015). Table 3-2 shows the average water quality parameters and their values during the 35 days of operation.

Table 3-2: Average water quality parameter values measured in the aquaponic sump through 35 days. MU-water = make up water added to the system. Fw = Fish waste. UF = Uneaten Feed.

Time	T Air °C	RH %	T water °C	O_2 mg/L	pH	Added $CaCO_3$ g	Added MU-water (L)	Total Fw and UF (kg)
35 d	14,10	80,22	16,55	8,95	6,74	200,00	697,50	378,90

3.2. Plant growth and development of ‘Frillice’ in monoculture

The monoculture study was conducted in a cold water aquaponic system in continuous operation. Four trays, each containing 24 ‘Frillice’ plants for a total of 96 plants, were harvested at approximately one week intervals. The time of harvest depended on the size of the plants. New seedlings replaced the harvested plants in the aquaponic system, and seeds were sown to provide fresh seedlings to replace the harvested lettuce.

3.2.1. Plant crop selection

Earlier experiments in done the Landvik aquaponic system concluded that the lettuce cultivar ‘Frillice’ was showed satisfactory growth in this particular system (Skar et al., 2015). This monoculture study of ‘Frillice’ was part of an experiment including biomass production of both lettuce and trout. The choice of plant crop was therefore predetermined to be ‘Frillice’.

3.2.2. ‘Frillice’ production from sowing to harvesting

The first step in the aquaponic plant production was preparing rockwool cubes and ‘Frillice’ seeds for germination. This was done by covering the floor of a plastic crate with approximately 120 rockwool cubes (5 x 5 cm) from Grodan. The crates were slowly submerged in the aquaponic water so that the cubes became saturated with water from the bottom up, removing air pockets from within. The wet cubes were placed on a rough meshed grate resting 2-3 mm above the water level and covered with a fiber cloth to ensure high relative humidity (Figure 3-3). This would ensure high germination success. The germination area was equipped with an air pump with an air stone that aerated the water taken from the aquaponic system.


Figure 3-3: A picture showing the germination area with 'Frillice' seedlings of different ages. The white fiber cloth covers newly sown seeds.

Seedlings could absorb nutrients from beneath the grate. The water was heated by a 300 W (Regelheizer RH01-300) aquarium water heater from SMF Aquaristik, and was refilled as needed. The seeds were pushed down into pre-made holes in the cubes, until resistance was felt. The sowing depth was approximately 1-1.5 cm and the plants were left to germinate in natural light. The rockwool cubes were checked for roots penetrating the cubes one week after sowing. The cubes with a few, straight roots were placed into the aquaponic system when the roots of the seedlings were 2-3 cm. The seedlings with several thin roots were placed into the aquaponic system after the healthier ones because they were of sub-optimal quality. A few, long and straight roots were optimal for aquaponic plant production compared to bushy and shorter roots.

The 'Frillice' plants were placed into the aquaponic system once their roots were more than 3 cm long and reached the water beneath the plant trays that the plants were floating in. Plant trays made of Styrofoam kept the plants in place with plastic frames that held the rockwool cubes suspended 3 centimeters above the water. This small gap between the rockwool cube and the aquaponic water reduced fungal growth because the cubes were kept dry. Four plant trays, each containing 24 plants, were harvested weekly or less frequently depending on their size. The plants grew in the aquaponic system for a total of 3-4 weeks until they were large enough to be harvested. The harvested lettuce was quickly replaced by new seedlings. This experiment was conducted through 6 weeks, allowing two full crop cycles from sowing to harvesting to be completed. The staggered plant production was maintained to keep the balance between nutrient production by fish and nutrient uptake by plants between the preliminary and comparative study.

3.2.3. Plant sampling and data collection

Four plants were randomly chosen from each plant tray at the time of harvest and used for data collection. The weights of another four randomly chosen plants were measured once a week for six weeks, from the time they were put into the aquaponic system up until the time of harvest. This was done by cutting the rockwool cubes open and removing the plant completely from the rockwool cube, taking great care not to damage the fragile roots (Figure 3-4). It was important to separate the 'Frillice' plants from their rockwool cubes to ensure that the recorded data reflected the weight of the plants and not the rockwool. Four plants from four replicates, a total of 16 plants, were collected after the plants had been growing in the aquaponic system for four weeks. Leaves and roots were separated, chopped up and carefully mixed and put into plastic bags, one for the leaves and one for the roots. The bags were frozen at -18 °C an stored for later analysis of plant nutrients. The remaining 80 plants were removed from the aquaponic system and prepared for sale. Old or discolored leaves were removed from the marketable yield following visual inspection. The total yield was found by weighing the total lettuce harvest. Measurements of fresh weight per lettuce plant were not taken in this study, but were calculated by dividing the total harvest weight by 96 plants.


Figure 3-4: A picture of 'Frillice' when removed from the rockwool cubes, ready for weighing.

3.2.4. Fish culture and sampling

The fish species present in the system varied throughout the lettuce study. Rainbow trout (*Oncorhyncus mykiss)* and brown trout (*Salmo trutta*) were present in the system during the start of the experiment. The rainbow trout were harvested at September 14, 2015 the study due to their size and in preparation of introducing a new salmonid fish species locally known as bleke (*Salmo salar L.*) into the aquaponic system. Technical difficulties resulted in major fish mortalities in October 2015. The fish were replaced quickly after the incident preventing a total collapse of the nutrient dynamics in the aquaponic system. New one year old fish were acquired at an average weight of 100 grams from Syrtveit Fishery in Evje and transported to NIBIO Landvik. The aquaponic system was without fish for eight days. A total of 110 bleke and 106 brown trout were introduced to the aquaponic system, separated by species and evenly distributed into the two fish tanks per fish species. The fish were fed 50 – 100 g (Nutra RC 3mm Skretting AS) fish feed daily. NIBIO staff checked the tanks for mortalities every day.

There was no scheduled harvest of fish during the ‘Frillice’ study. The emergency harvesting of dead and dying fish was carried out to remove all the fish following the high mortality incident before replacing them with new fish in accordance with fish health regulations. Both dead and dying fish were removed with a handheld net. The dead fish were discarded while the dying fish were killed. A lethal dose of the sedative Benzokain was added to a 70 liter plastic tub filled with water, some airstones and an airpump for mixing. The dying fish were placed into the plastic tub and removed once they were sufficiently sedated before being killed, weighted and measured.

3.2.5. Water sampling

Water samples were taken from the sump once a week. The water was collected in one-liter plastic bottles from Biltema AS (Art. No. 36-1767) and stored at -18 °C.

3.3. Plant growth and development of lettuce, coriander and swiss chard in polyculture

3.3.1. Experimental set up and system modifications

The main experiment of this Masters' thesis was as a quantitative study consisting of a randomized control trial (RCT), investigating differences in plant growth and development between the Landvik aquaponic system and a hydroponic control. This experiment type was chosen to reduce the bias for either plant production system. The experimental set-up with the placement of all randomized replicates can be found in Appendix A.

The plant growth parameters were generally chosen based on parameters used to describe growth and development of plants produced investigated in aquaponic papers. These parameters should describe the growth and development of the plant species and cultivars as closely as possible. The literature study showed that yield per square meter and fresh weight per plant had been used in most of the aquaponic studies. Plant height was measured in lemon basil (Hanson et al., 2008) and coriander (Silva et al., 2015). The maximum leaf height of coriander and swiss chard was known to be higher than the height of lettuce, providing a describing growth parameter. Bathia & Wasiim (2012) measured shoot height and root length of spinach, basil and watercress. Root length was chosen as another parameter to support leaf height because it described the plant root growth and development. The leaf count parameter was chosen as it described the development and maturation of the plants thus providing data for a leaf count development graph. The number of plants per rockwool cube was a result of choosing not to remove additional seedlings from the coriander and swiss chard cultivars to see if the increased amount of plants could obtain satisfactory yields.

Minor changes were done to the aquaponic system in preparation for the comparative study. Chelated iron (Fe-DTPA) was added to reduce a suspected iron deficiency observed in strawberry (*Fragaria × ananassa*) plants that were used to screen for nutrient deficiency symptoms, while the test plants were still germinating. The strawberry plants were removed once the iron deficiency symptoms were gone. The amount of 'Frillice' present in the aquaponic system was reduced to make room for the other plant species. The pH-value was controlled by automatic additions of $CaCO_3$, before being changed to $K_2CO_3^-$ additions to reduce an observed build-up of sediments. The monoculture of 'Frillice' was maintained throughout the comparative study to balance the nutrient dynamics of the aquaponic system. The whole aquaponic plant beds were grown in a 'Frillice' monoculture except for the four plant trays containing the test plants for the comparative study. While the 'Frillice' plant production continued as a staggered monoculture production, the test plants used in the comparative study was grown as a batch production.

A fish tank of 170 liters, identical to the one used for the germination chamber served as a hydroponic control to the aquaponic comparative study. The electrical conductivity (EC) of the hydroponic control was maintained at approximately 1300 µS/cm by adding nutrient solution if the EC decreased too much. The nutrient solution used in the hydroponic control was made up of 0.5g of Christalon Indigo mixed with 0.5g of Cacinite for every one liter of water. This resulted in 85 g of each nutrient solution being mixed into 170 liters of the hydroponic control. Table 3-3 shows the nutrient concentrations of the hydroponic solution per liter.

Table 3-3: The combined nutrient concentration of 0.5 g Christalon indigo and 0.5 g Calcinite per liter of nutrient solution used in the hydroponic control. ppm = parts per million. Calculated from nutrient solution concentrations from Yara b.

ppm	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Mo	Zn
Per liter	120	24,5	123,5	95	21	28,5	0,135	0,02	1,0	0,3	0,02	0,135

3.3.2. Plant crop selection

The literature study showed that several growth factors influence the successful production of plants in aquaponic systems. Production of plants adapted to growing conditions that are similar to the ones in the cold water aquaponic system should grow better than other plants. The monoculture study of ‘Frillice’ showed another critical factor for successful production; sufficient root growth. Sub-optimal root growth would lead to plants suffering from drought stress because the roots would not reach down to the water, resulting in wilting. Previous experiments investigated the growth of plants such as lettuce, rucola (*Eruca sativa*), dill (*Anethum graveolens*), parsley (*Petroselinum crispum*) and swiss chard (*Beta vulgaris* ssp. *vulgaris*) in the NIBIO Landvik system (Skar et al., 2015). Plant cultivars and species were selected from a seed catalogue from LOG AS, based on potential commercial value and research interest. The Norwegian overview of fruits and vegetables (NOoFV) showed that coriander should be chosen due to it being the second most bought herb in Norway. Plant seeds should be organic to prevent chemicals from entering the aquaponic system. The lettuce cultivar ‘Frillice’ was chosen as a control. Three lettuce cultivars called ‘Frillice’, ‘Salanova Descartes’ and ‘Salanova Excite R2’, two swiss chard cultivars called ‘Bulls Blood’ and ‘Fordhook Giant’ as well as a coriander (*Coriandrum sativum* L.) cultivar called ‘Marino’ were used in the preliminary germination experiment. This ensured that the germination of plants from different species could be tested, providing important information on the early developmental growth of these plants in cold water aquaponic systems.

A preliminary study of germination success was conducted to determine which four plant cultivars and species would be chosen. Rockwool cubes were divided into six groups with each group consisting of one plant species or cultivar. The seeds for the coriander cultivar ‘Marino’ consisted of multiple plant seeds in each seed capsule. The swiss chard cultivars had multiple organic seeds in seed capsules. ‘Frillice’ was pelleted and organic, while ‘Salanova Descartes’ and ‘Salanova Excite R2’ were pelleted and chemically treated. Each of the six plant cultivars were sown with one seed or seed capsule per rockwool cube and treated similarly to ‘Frillice’ plants while they were germinating during the preliminary study. Artificial lighting with a photoperiod of 16 h on, 8 h off was added to the germination chamber to supplement natural sun light as the experiment progressed into autumn.

The germination experiment was repeated due to poor germination success in the first germination experiment. Sub-optimal moisture levels in the rockwool cubes was the suspected cause. 300 rockwool cubes were once again divided into six groups, one for each plant species and cultivar. The cubes were placed on shallow plant trays and top-irrigated without fiber cloth covering to reduce moisture levels compared to the first trial (Figure 3-5). Ten recently watered rockwool cubes with seeds of the lettuce cultivar ‘Salanova Excite R2’ were weighed and used to calculate the average weight of the wet cubes. Ten dry, empty rockwool cubes were weighed and used to calculate the average weight of the dry cubes, and the water volume used for irrigation was calculated by subtraction. The water volume used for each irrigation was approximately 3,464 liters, evenly distributed on all rockwool cubes. The total water volume per cube was 0,012 liters. Irrigation with less water compared to the monoculture study of ‘Frillice’ production lead to much higher germination success of the other plants without decreasing the germination success of ‘Frillice’. Although the germination time was only a few days, the root growth was slower. This time determined which plant cultivars and species would be included in the main experiment. The

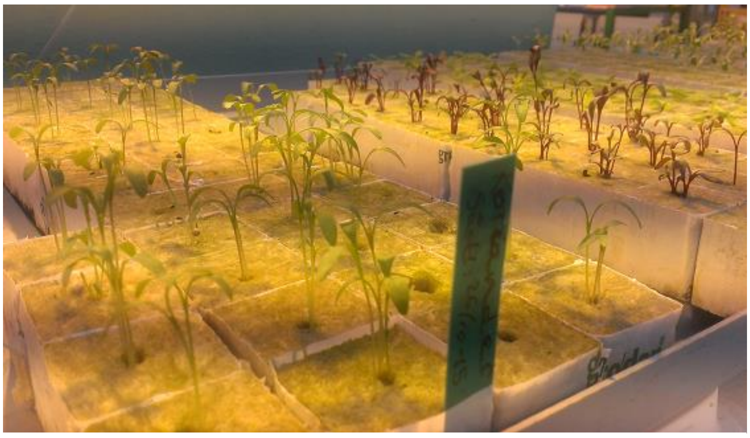


Figure 3-5: Drier germination conditions resulted in higher germination rates in the second germination trial compared to the first.

plant cultivars ‘Salanova Descartes’ and ‘Fordhook Giant’ were discarded, leaving ‘Frillice’, ‘Salanova Excite R2’, ‘Bulls Blood’ and ‘Marino’ to be tested in the experiment.

3.3.3. Plant production from seedling to harvesting

The four plant cultivars were moved from the germination chamber and placed into the aquaponic system and the hydroponic control on November 23, 2015. The transfer date was later than optimal due to a compromise between waiting for ‘Bulls Blood’ plants to develop sufficiently long roots and letting ‘Frillice’ outgrow all other plants. 9 plants of each cultivar were transferred into the hydroponic control, and divided into three replicates. 24 plants of each cultivar were transferred into the aquaponic system, and divided into four replicates. Plant placement was randomized within each replicate of the aquaponic system and the hydroponic control. Although the location of the different cultivars was completely randomized, the selection of individual plants was not random. This resulted in more vigorous and strong plants being placed into the hydroponic control because the plants with the best root growth were transferred into the hydroponic control first. Plants of subsequently lower root growth were put into the aquaponic system. The remaining ‘Bulls Blood’ plants with insufficient root growth caused a delay of one week between the first and the last introduction of ‘Bulls Blood’ plants into the aquaponic system.

The roots of different plants grew into each other and got entangled after some weeks of growth. This caused the root measurements to become less accurate as roots broke off during handling. Increased difficulties with root measurements, entanglement of leaves and roots, and a suspected nutrient deficiency or disease on ‘Marino’ resulted in coriander being harvested one week early. The other cultivars were harvested at 04.01.2016. The timeline of the comparative study is found in Table 3-4. The “X” shows when an activity was started as well as the duration of the activity. The preparation of rockwool cubes and sowing was done on the same day, while the germination and root growth period lasted more than two weeks for some cultivars, especially ‘Bulls Blood’. The plants were transferred into their respective systems within two weeks while data collection started on week 6 and continued until the final harvest in week 11 of the production cycle.

Table 3-4: The timeline of the comparative study. The "X" marks the week at which an activity was started.

Activity\week of production	0	1	2	3	4	5	6	7	8	9	10	11
Preparing rockwool-cubes	X											
Sowing	X											
Germination and root growth	X	X	X		X							
Transfer into growth systems						X	X*					
Data collection							X	X	X	X	X	X
Harvesting											X**	X

* = Bulls Blood seedlings were transferred over the course of two weeks. ** = Marino plants were harvested one week prior to the others.

3.3.4. Plant sampling and data collection

The plant cultivars grew in the aquaponic system and the hydroponic control from 23.11.2015 to 04.01.2016. The cultivars 'Bulls Blood' and 'Marino' only got 5.5 weeks of growth. 'Bulls Blood' was transferred into the systems from 23.11.2015 to 30.11.2015 while 'Marino' was harvested earlier. 'Frillice' and 'Salanova Excite R2' grew for 6.5 weeks. Growth parameters were measured six times during the experimental period. The total number of plants per rockwool cube and the leaf number were visually counted. Maximum leaf height was measured from the base of the leaves to the maximum height of the plants. Coriander and swiss chard leaves started to bend in the later weeks of the study. Their leaves were therefore straightened and measured as they stood. Plants were briefly lifted to allow measurements of the maximum root length of the primary root without looking at root system width, before putting the plants back in their respective growth systems. Weight measurements were taken when the plant cultivars and species were harvested, preventing unnecessary mechanical damage from handling. The average number of plants per rockwool cube was calculated after the harvest.

The method of harvesting plants was the same for all cultivars. All the plants of one cultivar were removed from their plant tray (replicate) at the same time. The individual plants were removed from their rockwool cubes and divided into two piles; one with leaves and stem, and one with roots. The leaves and stems were weighted on a Mettler Toledo PB3002-S balance and the yield of each plant cultivar was recorded before weighing the roots on the same balance. This was repeated for all replicates in both the hydroponic and the aquaponic systems. Each pile of plant parts was cut into small pieces and mixed before placing the plant yields of leaves and roots into separate plastic bags. This was repeated for all four plant cultivars in each of the four aquaponic replicates, and each of the three hydroponic replicates. A total of 32 plastic bags from the aquaponic system and 24 bags from the hydroponic control were frozen at -18 °C.

3.3.5. Managing pests

The plants in the aquaponic facility suffered from different pests during the experimental period. A severe aphid infestation in the monoculture of 'Frillice' occurred during the seedling stage of the plants used in the comparative study and was of concern. Seedlings were very fragile during this stage of development because they had not yet managed to develop defenses to fight off pests. They were also placed very close to each other while waiting for leaf and root growth to become optimal for plant transfer into the aquaponic and hydroponic systems and this made it easy for aphids to colonize the test plants. Aphids were removed and killed manually while yellow glue traps were used to control flying pests before the biological control agents (beneficial insects) could be placed into the plant crops. The parasitic wasp (*Dacnusa sibirica*) from Koppert was used to control crop damage caused by aphids (*Aphidoidea* sp.) and miner flies (*Liriomiza trifolii*, *Liriomiza bryoniae* and *Liriomiza huidobrensis)*. Predatory mites (Hypoaspis miles/Stratiolaelaps scimitus) from BioProduction were used primarily to control fungus gnats (*Mycetophilidae* sp.) (Seljåsen, R. & Skar, S. L. G. personal communication, December 2015).

3.3.6. Fish culture and sampling

The fish that were restocked during the monoculture experiment with 'Frillice' grew throughout the comparative study. Fish data was gathered at the end of the experimental period (05.–06.01.2016). No statistical investigation of the difference in yield and size of fish was done. Fish harvesting was done under the supervision of an aquaculture specialist and a veterinary. The procedure for catching and bleeding the fish was identical to the procedure mentioned earlier, except for a longer waiting time before the fish became lethally sedated due to increased amounts of fish being sedated. A lethal dose of Benzokain was made by mixing 100 g of Benzokain into a 70 liter plastic tub filled with water, complete with airstones and an airpump. Handheld nets were used to catch fish after the solution had been mixing for approximately 10 minutes. Batches of around 20-30 fish were caught and put into the plastic tub where they became sedated after a few minutes. The fish were removed one at a time and each one was visually inspected to look for disease or injuries of their mouth, gills, fins and skin before they were killed. Using both a lethal dose and bleeding ensured that fish would not awake from sedation. The fish length and weight was measured on a Mettler Toledo PB3002-S balance. This procedure was repeated until all fish were harvested. Three individuals of both brown trout and bleke were taken from the harvest and frozen whole at -18 °C for nutrient analysis.

3.4. Analyses of plants, fish, water and sludge in polyculture

3.4.1. Water quality parameters

The water temperature was recorded using a temperature sensor connected to a temperature logger system. The temperature sensor was located in the aquaponic sump and recorded the temperature of the system as part of the general water quality control system of the aquaponic facility. When conducting the comparative study, three extra temperature sensors were added. One sensor was placed in the aquaponic DWC bed and one in the hydroponic control, while the third sensor was used to record the air temperature in the greenhouse. pH was measured automatically by a pH-element from Hamilton Polilyte Plus 120 in the sump of the aquaponic system, connected to a signal converter from Knick Pikos, an M-System M2XU Universal Transmitter, and a logger that displayed the pH (Skar et al., 2015). Manual pH measurements were done in the hydroponic control and the aquaponic DWC beds in the comparative study. Dissolved oxygen (DO) was measured in the sump of the aquaponic system and recorded daily by the automatic water quality control system. DO and EC was also measured manually in the aquaponic and hydroponic DWC beds by a DO-meter from OxyGuard Handy Polaris and a HI 983303 conductivity-meter from Hanna Instruments.

Water sample collection was done on a weekly basis, similarly to what was done in the monoculture study. Aquaponic sludge, water with uneaten fish feed and fish feces, was sampled in a similar plastic bottle used for water sampling. The aquaponic sludge was collected from an IBC-tote used for sludge collection after mixing the sludge to get a more homogenous and representative sample. The sludge sample was taken after the end of the comparative study. All samples were stored at -18 °C.

3.4.2. Preparation of plant, fish and water samples

Frozen plant samples were sent to NMBU to be processed. A Mettler Toledo PB3002-S balance was used to measure approximately 200 g of fresh plant material from the samples. The plant samples were then put into plastic net bags and placed in a heating chamber from Termaks AS at 58 °C for a minimum of 48 hours, until all samples were dry enough for milling. The dry weight of the samples were recorded using the same balance as before.

Dried plant material was first milled in a coarse HR1629/00 Philips hand blender and later in a fine Krups KM75 coffee mill, turning the plant material into fine powder. The plant samples of leaves and roots that were originally kept separate, were now combined and mixed into 50 mL plastic centrifuge tubes from VWR.

3.4.3. Analyses of plant, fish and water samples

The processed plant samples were analyzed for total N, P, K, Ca, Mg, Fe, Cu, Mn, Na and Zn through Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) at the Department of Environmental Science at NMBU. Some water analyses were done weekly at the aquaponic facility at NIBIO Landvik with test kits from Hach Lange. Aquaponic water was tested for P, NH_4^+, NO_2^-, NO_3^- and Cl. These water tests were analyzed in a DR3900 Benchtop Spectrophotometer from Hach Lange. Frozen water samples that were collected earlier during the comparative study were sent to NMBU for additional analysis of total N, P, K, Ca, Mg, Fe, Cu, Mn, Na and Zn. All nutrient analysis values were based on air dried samples except for total N that was dried at 55 °C prior to the analysis. The aquaponic sludge sample was sent to Eurofins Environment Testing Norway AS where the sample was analyzed for suspended solids, total P and total N. The methods used for analysis were NMKL No 161 1998 (Atom Absorption Spectrophotometry) mod for P, NMKL 6 (Kjeldahl) for N and NMKL 23 (Gravimetric method) for suspended solids.

3.5. Statistical analyses

Microsoft Excel (2013) was used for data collection. Minitab (version 16) was used to conduct the analysis of variance (ANOVA) (General Linear Model procedure). Microsoft Excel (2013), One-way ANOVA and Tukey’s pairwise comparison test at $p<0.05$ were used to obtain significant differences. Microsoft Excel was used to present the data graphically.

4. Results

4.1. Plant growth and development of ‘Frillice’ in monoculture

4.1.1. Total yield and fresh weight of ‘Frillice’ plants

The monoculture study of ‘Frillice’ showed that the total yield varied throughout the experimental period (Figure 4-1). The highest yield of fresh lettuce leaves was obtained in harvest from 28.08.2015, totaling 19.54 kg. The average individual plant weight varied between 102.29 g in the smallest harvest and 203.55 g in the largest harvest. The highest fresh weight of roots was found in the same harvest of the largest yield, with a total root weight of 1.99 kg. Both graphs seem to follow a similar trend with largest and smallest harvest weights of both leaves and roots being on the same harvest dates. The yield of ‘Frillice’ leaves was calculated to vary between 3436.94 g – 6839.28 g/m^2 based on a plant density of 33.6 plants per m^2.

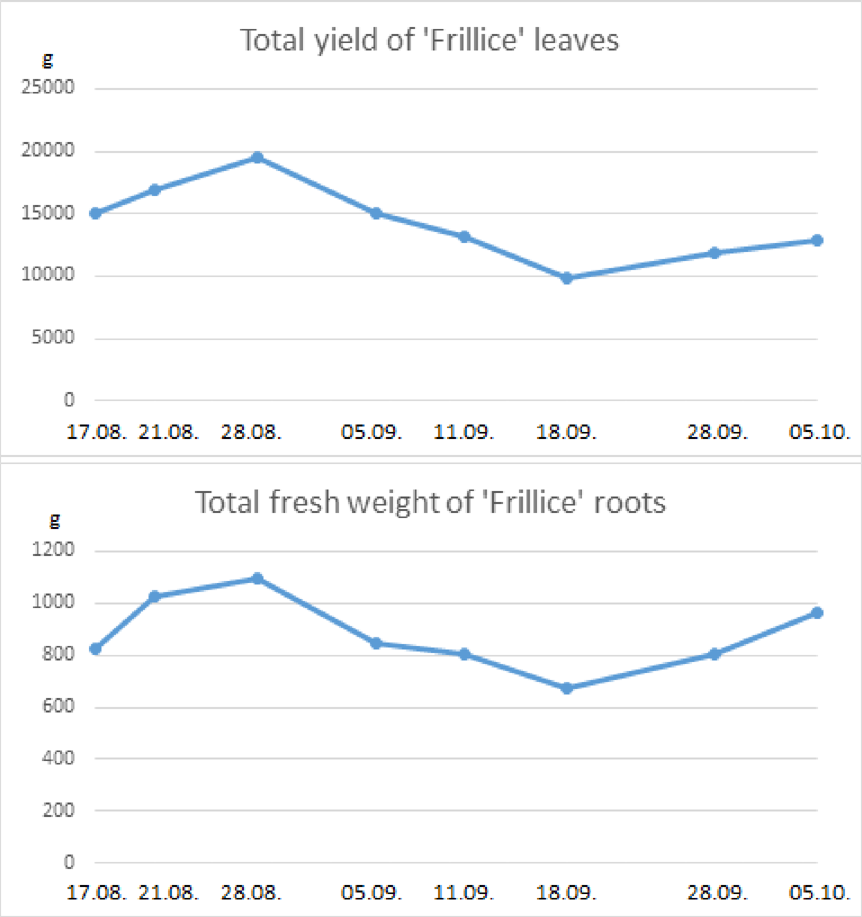


Figure 4-1: Changing yields from August 2015 to October 2015 (top) and the changing weight of ‘Frillice’ roots (bottom).

4.2. Nutrient content of 'Frillice' plants and aquaponic water

4.2.1. Nutrient content of 'Frillice' plants

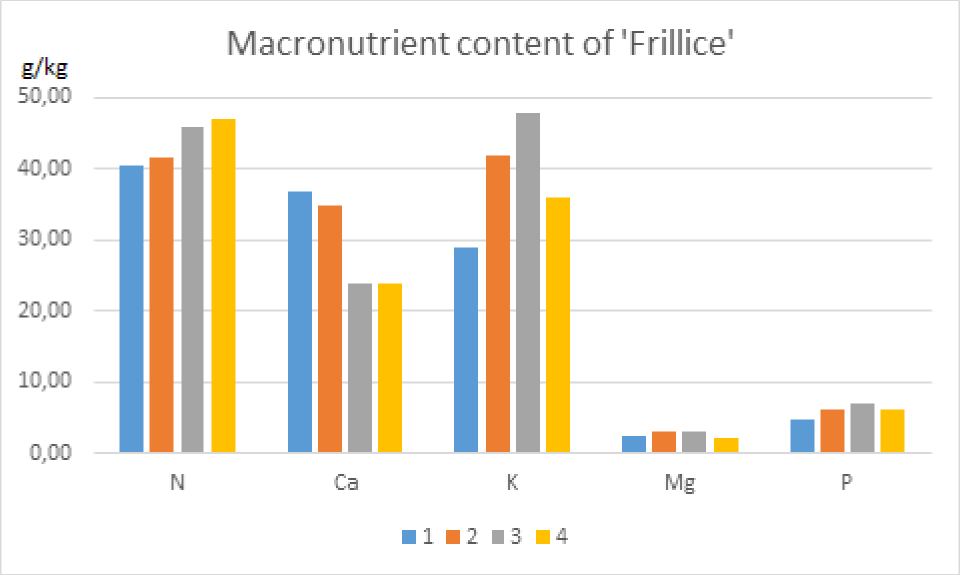


Figure 4-2: Nutrient content of macronutrients measured in four replicates (1-4) of 'Frillice'. The values are based on dry weight.

The nutrient analysis showed individual variations between the replicates for all measured nutrients. Figure 4-2 shows the variation in macronutrient content. Plants from replicate 3 was found to have the highest amount of Cu, Fe, K, Mg and P (Table 4-1). Mn content was higher in plants from replicates 1 and 2 compared to 3 and 4. Plants from replicate 2 had three times as high Na values (8.7) as replicates 1 and 4 (2.9), while the Zn content was slightly higher in plants from replicate 1 than the other three.

Table 4-1: An overview of the macro- and micronutrient content of four replicates of 'Frillice' and their average values, harvested on the same date (n= 16).

Replicate No.	**Macro- and micronutrients (g/kg) dry weight**									
	N	**Ca**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Na**	**P**	**Zn**
1	40,62	37	0,0075	0,089	29	2,5	0,059	2,9	4,8	0,14
2	41,52	35	0,0088	0,13	42	3,1	0,048	8,7	6,1	0,11
3	45,81	24	0,011	0,13	48	3,1	0,034	3,9	6,9	0,11
4	47,15	24	0,01	0,093	36	2,1	0,034	2,9	6,1	0,12
Average	43,78	30	0,0093	0,11	38,75	2,7	0,044	4,6	5,98	0,12

4.2.2. Nutrient concentration of aquaponic system water

Water samples were taken before, during and after the experimental period of the preliminary monoculture, lasting from 18.08 – 05.10. The plants spent approximately four weeks in the aquaponic system before being harvested, depending on when they reached a harvestable size. The nutrient concentration varied between the three sampling dates (Table 4-2). Total nitrogen varied between 91-120 mg/L and Ca varied between 140-180 mg/L, both having the lowest concentrations in September. The concentrations of Total N, Ca, Cu, Mg, Na and Zn all suggest a weak trend of high content in the first sampling date, lower in the second, and increasing content at the last sampling date. Fe and Mn decreased during the period while K content was below detection levels.

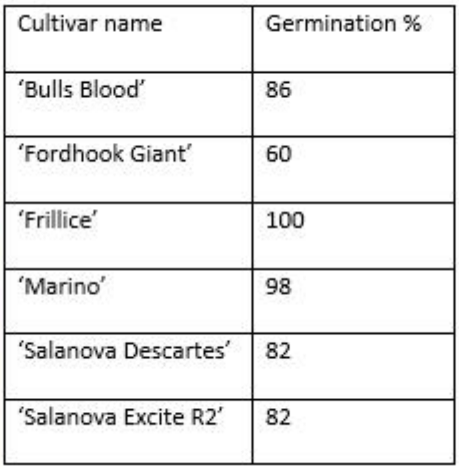
Table 4-2: Changes in the nutrient concentration of the aquaponic system water. The water samples were taken from the sump. Calcium was added as $CaCO_3$ to control the pH-level of the water. TOT-N-values are based on one sample, while the rest are based on two samples. TOT-N = Total nitrogen.

	Nutrient concentration (mg/L)									
Sampling date	TOT-N	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
14.08 -15	110	175	0,019	0,0245	<0,51	4,1	0,00325	17	3,7	0,014
25.09 -15	91	140	0,015	0,011	<0,51	3,4	0,0016	14	5,1	0,012
20.10 -15	120	180	0,018	0,0092	<0,51	4,6	0,0016	19,5	5,1	0,013

4.3. Plant growth and development of lettuce, coriander and swiss chard in polyculture

4.3.1. Germination and root growth of seedlings

The first germination experiment resulted in poor germination success (data not shown). The second germination experiment resulted in a higher germination success rate, although the initial root growth was slow. The swiss chard cultivar ‘Bulls Blood’ was used to illustrate the slow root growth that occurred in cultivars ‘Bulls Blood’, ‘Fordhook Giant’ and ‘Salanova Descartes’ (Figure 4-3). High germination success rate was not enough to predict the growth and development of cultivars. They also need to show satisfactory root development.



Cultivar name	Germination %
‘Bulls Blood’	86
‘Fordhook Giant’	60
‘Frillice’	100
‘Marino’	98
‘Salanova Descartes’	82
‘Salanova Excite R2’	82

Figure 4-3: The result of optimal and poor root development of 'Bulls Blood' (left) and the germination success % from the second germination experiment (right).

4.3.2. Plant yield and growth

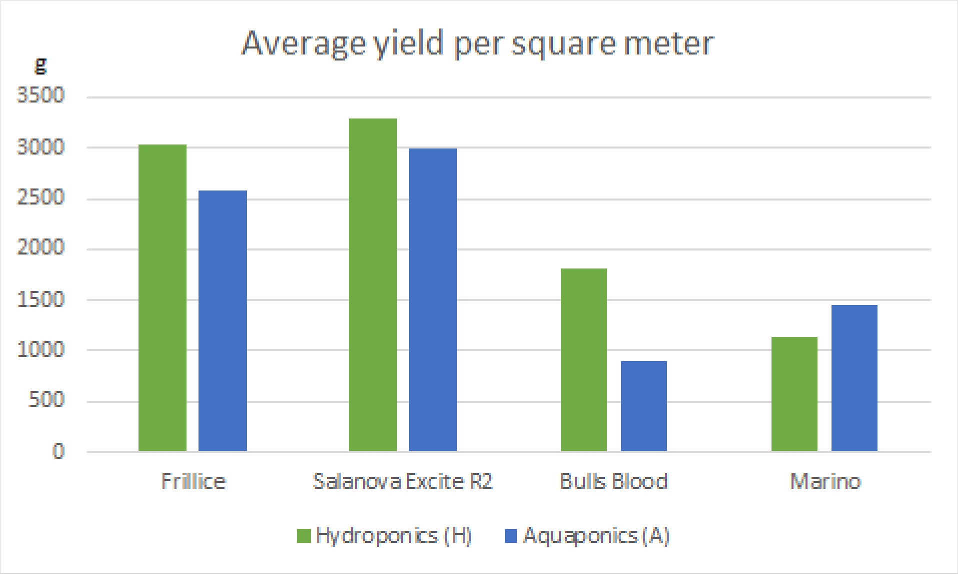


Figure 4-4: Average yields of hydroponic and aquaponic plants per square meter. The hydroponic plants were grown at a higher plant density (36/m^2) than the aquaponic plants (33.6/m^2).

All plant cultivars except ‘Marino’ seemed to produce a lower average yield/m^2 when grown in the aquaponic system as compared to the hydroponic control (Figure 4-4). This trend was not statistically significant as ANOVA showed no significant difference between the average yield of cultivars and the aquaponic and hydroponic systems ($p<0.05$) using the General Linear Model procedure.

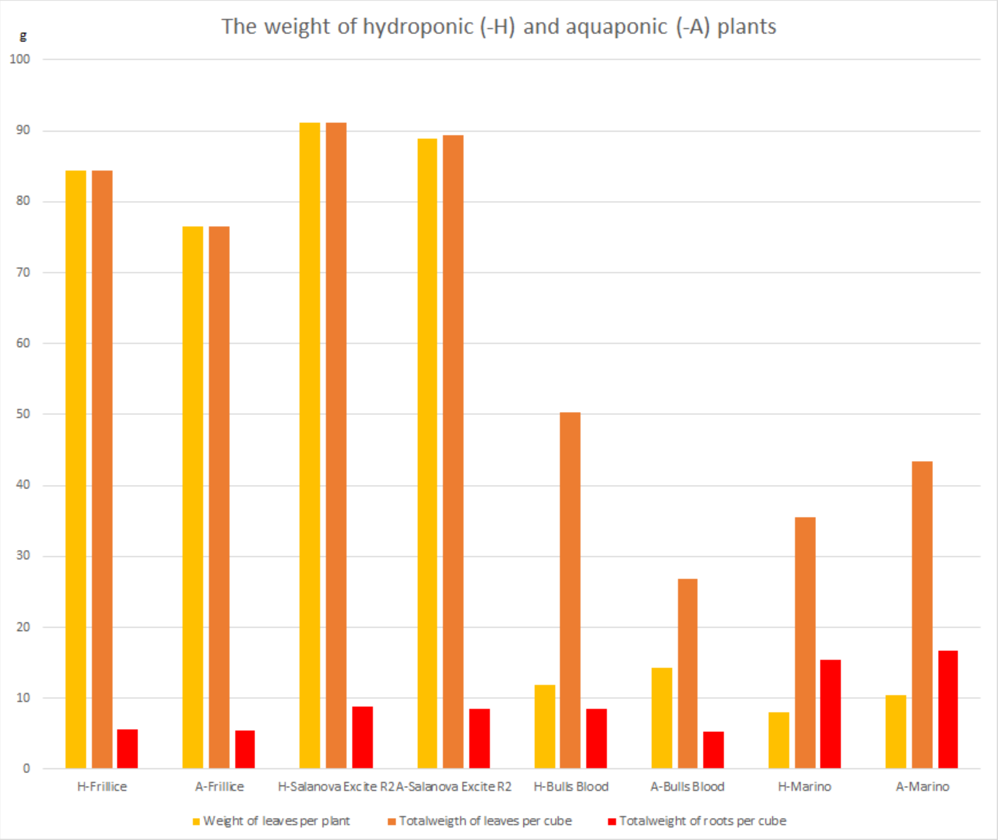


Figure 4-5: The average fresh weights of hydroponic (H-) and aquaponic (A-) plants, separated into weight of leaves per plant, total weight of leaves per rockwool cube, and total weight of roots per cube.

Table 4-3: Tukey's Method and 95,0 % confidence interval shows the leaf weight per plant, leaf weight per rockwool cube and root weight per rockwool cube of plants from the aquaponic system. Means that do not share a letter are significantly different.

Parameter	Plant cultivar	N	Mean (g)
Leaf weight per plant	'Frillice'	33	80,456a
	'Salanova Excite R2'	33	90,081a
	'Bulls Blood'	21	13,024b
	'Marino'	33	9,263b
Leaf weight per cube	'Frillice'	33	80,46a
	'Salanova Excite R2'	33	90,08a
	'Bulls Blood'	21	38,59b
	'Marino'	33	39,44b
Root weight per cube	'Frillice'	33	5,532b
	'Salanova Excite R2'	33	8,614b
	'Bulls Blood'	21	6,881b
	'Marino'	33	16,070a

A possible trend showing that ‘Frillice’ and ‘Salanova Excite R2’ obtained a higher leaf fresh weight per plant in the hydroponic control than in the aquaponic system was observed. A possible opposite trend was observed for ‘Bulls Blood’ and ‘Marino’. ANOVA showed no significant difference that could support that there was a difference between aquaponic and hydroponic systems ($p<0.05$). Significant differences were found between the average fresh weight per plant and plant cultivars. ‘Salanova Excite R2’ and ‘Frillice’ obtained a significantly higher leaf weight than ‘Bulls Blood’ and ‘Marino’ (Figure 4-5, Table 4-3). There were no significant difference between ‘Salanova Excite R2’ and ‘Frillice’, or between ‘Bulls Blood’ and ‘Marino’. 71.05 % of the variation in leaf weight per plant was explained by the General Linear Model.

A possible trend showed that the hydroponic control resulted in higher leaf weights per cube for all cultivars except ‘Marino’. ANOVA showed no significant difference between the aquaponic system and the hydroponic control ($p<0.05$). The leaf weight per cube followed the same trend as leaf weight per plant, with ‘Frillice’ and ‘Salanova Excite R2’ obtaining significantly higher leaf weights per cube than ‘Bulls Blood’ and ‘Marino’ ($p<0.05$). ANOVA showed no significant difference between the weights of ‘Salanova Excite R2’ and ‘Frillice’, or between ‘Bulls Blood’ and ‘Marino’. 43.86 % of the variation in leaf weight per rockwool cube was explained by the General Linear Model.

There was no observed difference between the root weights of ‘Frillice’ and ‘Salanova Excite R2’ grown in the aquaponic system and the hydroponic control. Aquaponic ‘Bulls Blood’ seemed to have a higher root weight than hydroponic ‘Bulls Blood’, while aquaponic ‘Marino’ seemed to have slightly lower root weight than hydroponic ‘Marino’. These trends were non-significant because ANOVA showed no significant difference between the average fresh weight of plant roots per rockwool cube and the aquaponic and hydroponic systems ($p<0.05$). Statistical differences were found between the average fresh weight of plant roots per rockwool cube and plant cultivars ($p<0.05$). The roots of ‘Marino’ were significantly heavier than the other plant species and cultivars ($p<0.05$). ‘Salanova Excite R2’, ‘Bulls Blood’ and ‘Frillice’ were not significantly different from each other. 45.79 % of the variation in root weight per rockwool cube was explained by the General Linear Model.

4.3.3. Leaf count development

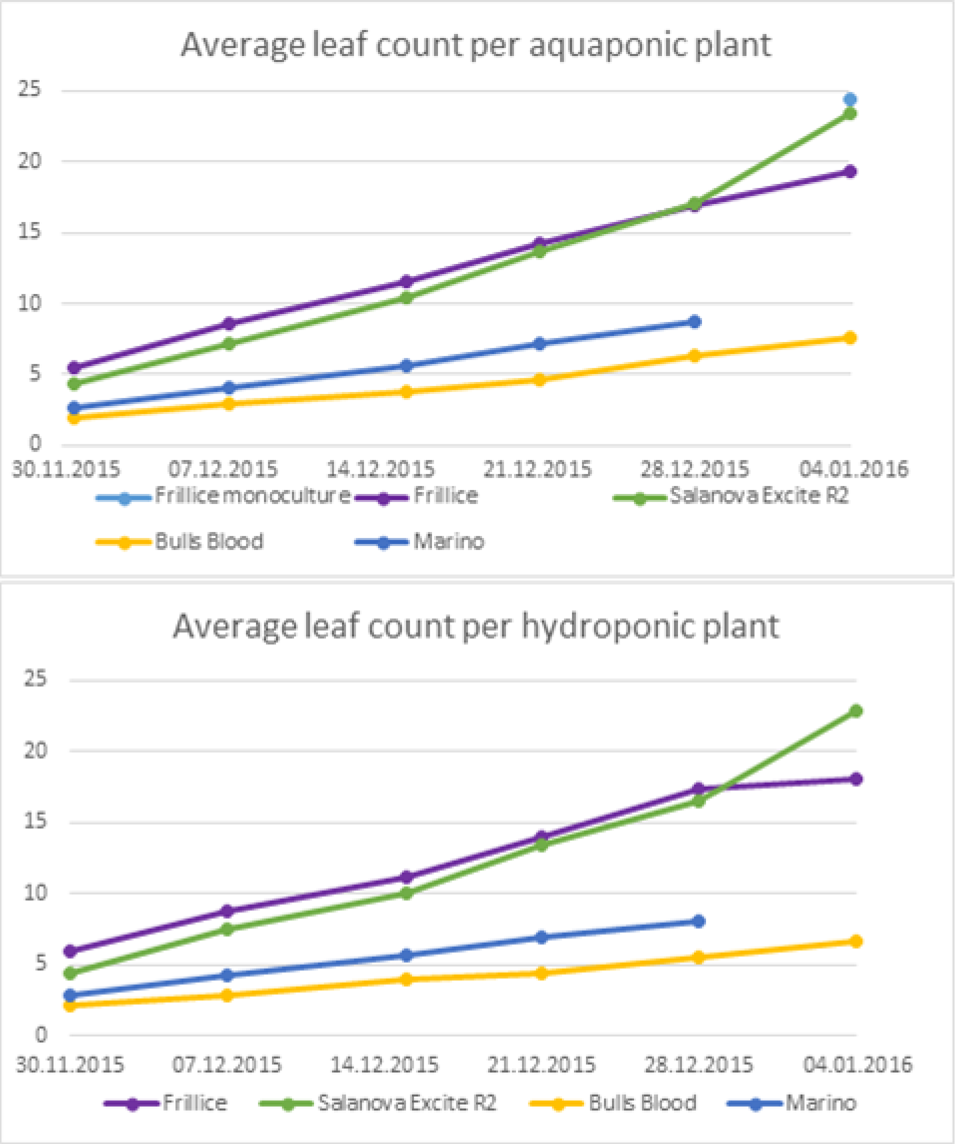


Figure 4-6: The average leaf count per aquaponic (top) and hydroponic (bottom) plant cultivar during six weeks of growth. The coriander cultivar 'Marino' was harvested one week earlier. The light blue dot at the top right in the aquaponic graph is the leaf count of 'Frillice' grown in monoculture during the comparative study.

Table 4-4: Tukey's Method and 95.0 % confidence interval showing the mean leaf count at harvest. Means that do not share a letter are significantly different.

Plant cultivar	N	Mean leaf count
'Frillice'	33	18,70b
'Salanova Excite R2'	33	23,19a
'Bulls Blood'	21	7,11c
'Marino'	33	8,40c

ANOVA showed no significant difference between the average leaf count of aquaponic and hydroponic treatments ($p<0.05$). Significant differences were found between the average leaf count and plant cultivars. 'Salanova Excite R2' had a significantly higher number of leaves than 'Frillice', at 23.2. 'Frillice' had a significantly higher number of leaves than 'Bulls Blood' and 'Marino', at 18.7. There was no statistically significant difference between the average leaf number of 'Bulls Blood' and 'Marino' (Figure 4-6, Table 4-4). 85.51 % of the variation in leaf count was explained by the General Linear Model.

4.3.4. Leaf height development

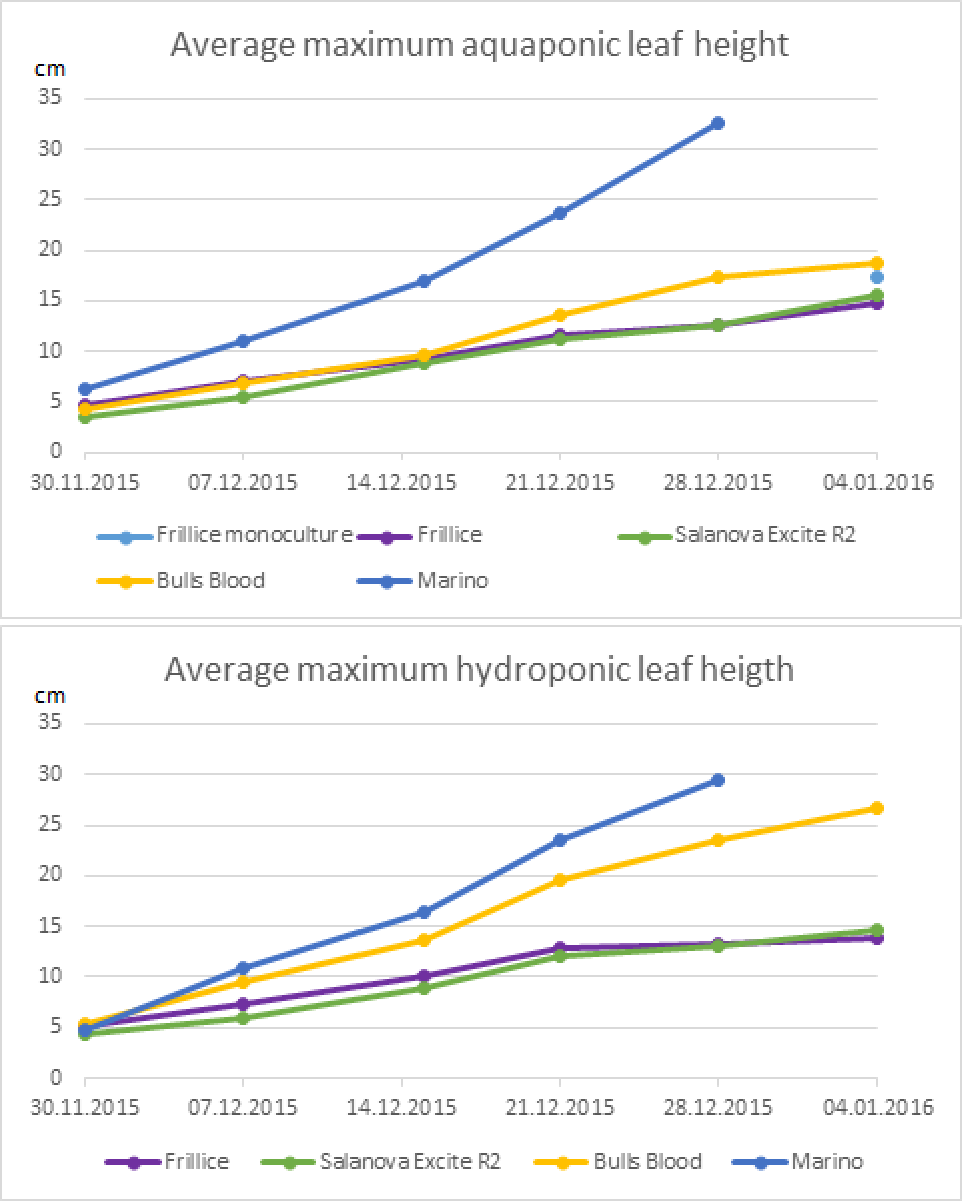


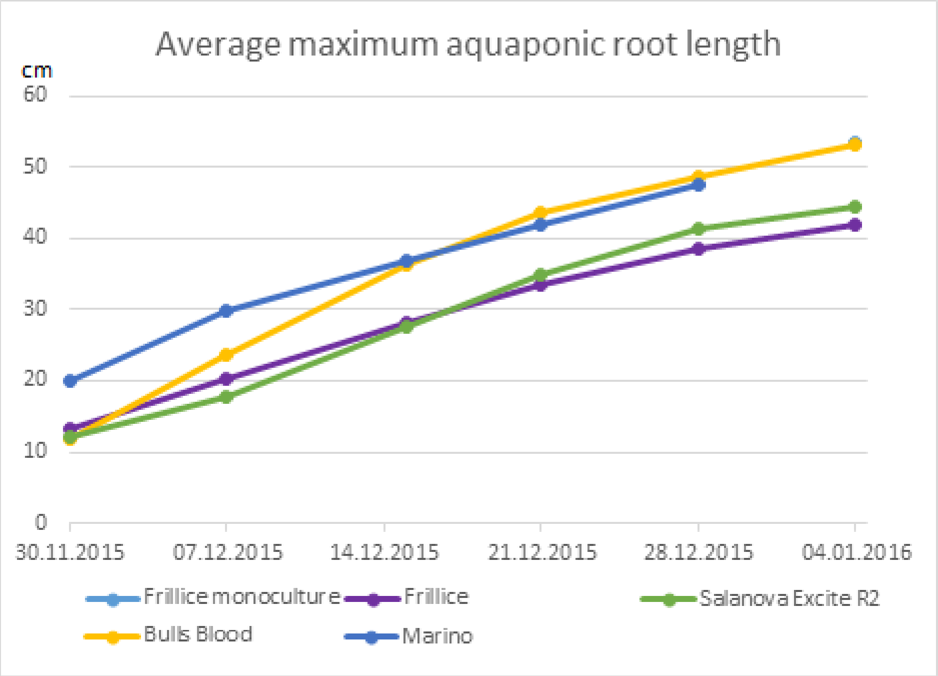
Figure 4-7: The change in maximum leaf height of aquaponic (top) and hydroponic (bottom) plants during six weeks of growth. The coriander cultivar 'Marino' was harvested one week earlier. The light blue dot at the top right in the aquaponic graph is the leaf height of 'Frillice' grown in monoculture during the comparative study.

Table 4-5: Tukey's Method and 95.0 % confidence interval showing leaf height at harvest. Means that do not share letters are statistically different.

Plant cultivar	N	Mean (cm)
'Frillice'	33	14,30c
'Salanova Excite R2'	33	15,10c
'Bulls Blood'	21	22,67b
'Marino'	33	31,02a

ANOVA showed no significant difference between the average maximum leaf height of aquaponic and hydroponic treatments ($p<0.05$). Significant differences were found between the average maximum leaf height and plant cultivars. 'Marino' and 'Bulls Blood' had significantly longer leaves than both 'Salanova Excite R2' and 'Frillice'. 'Marino' had the longest leaves at 31.0 cm. There was no statistical difference in leaf length between Salanova Excite R2 and Frillice (Figure 4-7, Table 4-5). 83.76 % of the variation in leaf height was explained by the General Linear Model.

4.3.5. Root length development



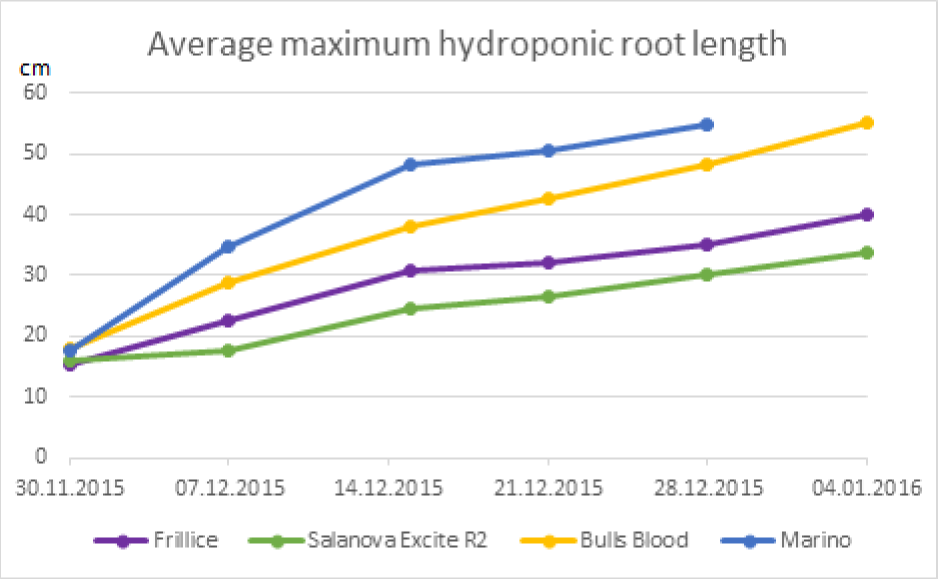


Figure 4-8: The change in maximum root length of aquaponic (top) and hydroponic (bottom) plants during six weeks of growth. The coriander cultivar 'Marino' was harvested one week earlier. The light blue dot, just behind the yellow 'Bulls Blood' dot at the top right in the aquaponic graph shows the root length of 'Frillice' grown in monoculture during the comparative study.

Table 4-6: Tukey's Method and 95.0 % confidence interval showing the mean root length at harvest. Means that do not share a letter are significantly different.

Plant cultivar	N	Mean (cm)
'Frillice'	33	40,96b
'Salanova Excite R2'	33	39,16b
'Bulls Blood'	21	54,12a
'Marino'	33	51,16a

ANOVA showed no significant difference between the average maximum root length of aquaponic and hydroponic treatments ($p<0.05$). Significant differences were found between the average maximum root length and plant cultivars. 'Bulls Blood' and 'Marino' had significantly longer roots than both 'Salanova Excite R2' and 'Frillice'. There was no significant difference in root length between 'Bulls Blood' and 'Marino', nor between 'Salanova Excite R2' and 'Frillice' (Figure 4-8, Table 4-6). 18.12 % of the variation in root length was explained by the General Linear Model.

4.3.6. Fish yield and size

Table 4-7: An overview of growth parameters of bleke and trout growing in four fish tanks. STD = Standard deviation. SGR = Specific growth rate.

	Fish tank 1		Fish tank 2		Fish tank 3		Fish tank 4	
Fish species	Bleke		Bleke		Trout		Trout	
Parameter	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight	Length	Weight	Length
Average	129,66	22,29	115,08	21,84	175,42	23,26	179,91	23,56
STD	36,18	1,56	25,19	1,29	70,50	2,57	64,84	2,54
Number of fish	41	41	47	47	53	53	53	53
Largest	205,3	25,5	165,7	24,4	490,4	32	426	31
Smallest	70,6	18	70,9	19,5	92,7	19	83	18
SGR	0,22		0,08		0,34		0,37	

The average weights of the two fish species were 122.37 g (bleke) and 177.67 g (trout). The weight ranged from 70.6 g – 205.3 g (bleke) and from 83.0 g – 490.4 g (trout). Their average sizes were 22.07 cm (bleke) and 23.41 cm (trout). The specific growth rate of bleke was 0.22 in fish tank 1 and 0.08 in fish tank 2. The specific growth rate of trout was 0.34 in fish tank 3 and 0.37 in fish tank 4. The values and calculations are based on data found in Table 4-7.

4.3.7. Nutrient content of plants

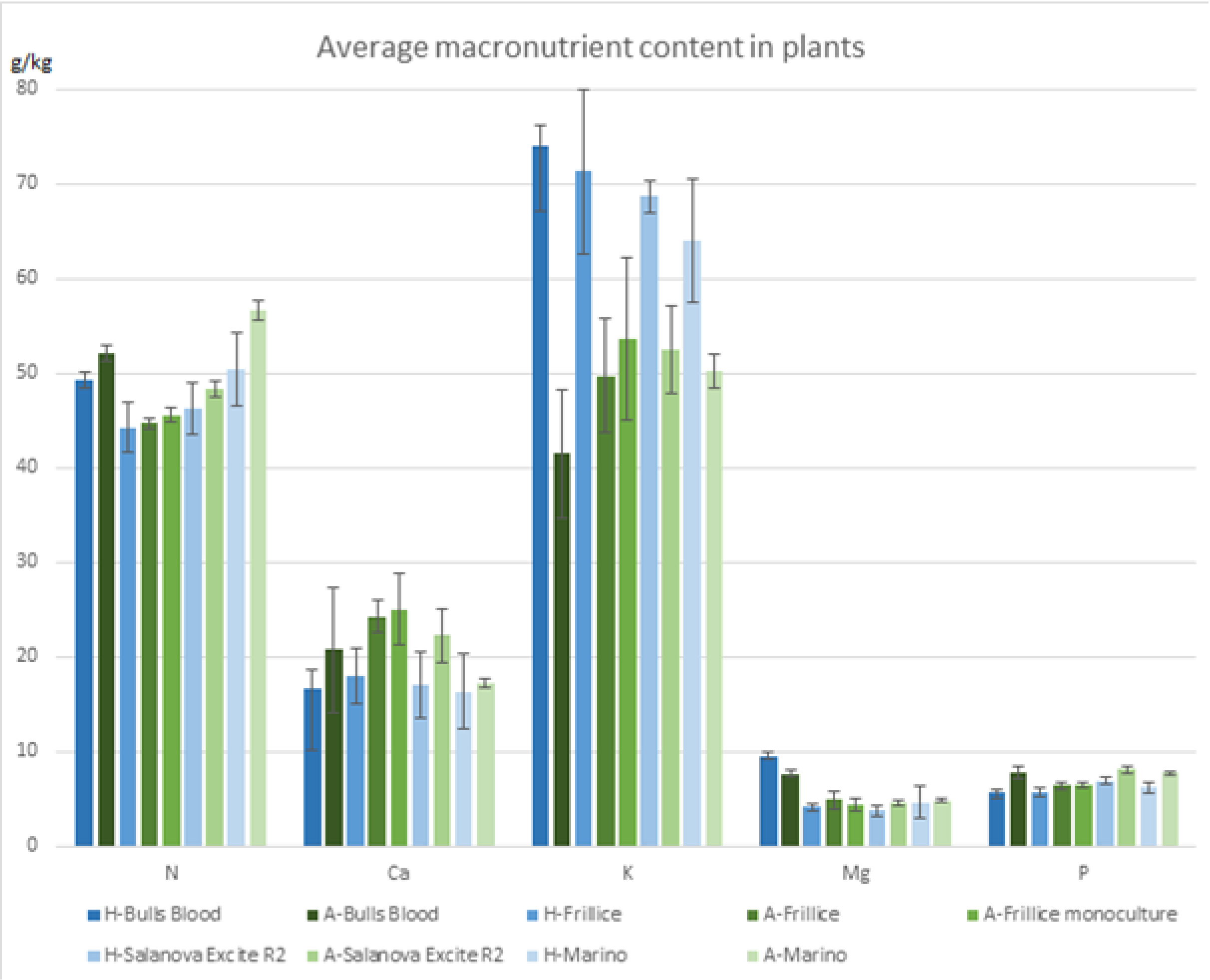


Figure 4-9: The nutrient content of the macronutrients found in blue graphs of hydroponic (H-) and green graphs of aquaponic (A-) plants. The vertical lines on the graphs show the standard error at p<0.05. Lines that do not overlap are statistically different.

The nutrient analysis of plants showed a high degree of variation between the plant species, cultivars and system (Figure 4-9). Aquaponic ‘Bulls Blood’ and ‘Marino’ had a higher nitrogen content when compared to their hydroponic counterparts. All other cultivars showed no significant difference in nitrogen content between systems. Aquaponic ‘Frillice’ had a higher Ca content than hydroponic ‘Frillice’, while all other cultivars showed no significant difference. The K content of all cultivars were significantly higher in the hydroponic control than in the aquaponic system. Hydroponic ‘Bulls Blood’ had a significantly higher Mg content than aquaponic ‘Bulls Blood’ while other cultivars showed no significant difference between systems. The P content of aquaponic ‘Bulls Blood’, ‘Salanova Excite R2’ and ‘Marino’ were all higher than their hydroponic counterparts. Only ‘Frillice’ showed no significant difference in P content between systems. The complete results of both macro- and micronutrient content from the nutrient analysis are shown in Table 4-8.

Table 4-8: Average values of macro- and micronutrient content of aquaponic (A-) and hydroponic (H-) plants in dry weight. A-Frillice monoculture = monoculture ‘Frillice’ grown in the same system that the polyculture ‘Frillice’ was grown in (n=3). Sample size of A-plants = 4. Sample size of H-plants = 3.

Plant cultivars	N g/kg	Ca g/kg	Cu g/kg	Fe g/kg	K g/kg	Mg g/kg	Mn g/kg	Na g/kg	P g/kg	Zn g/kg
A-Frillice monoculture	45,58	25	0,02	1,43	53,67	4,4	0,11	5,1	6,47	0,07
A-Frillice	44,71	24,25	0,02	1,81	49,75	4,85	0,13	5,38	6,35	0,07
H-Frillice	44,29	18	0,014	1,47	71,33	4,1	0,16	1,82	5,67	0,08
A-Salanova Excite R2	48,37	22,25	0,02	1,65	52,5	4,48	0,12	5,2	8,08	0,06
H-Salanova Excite R2	46,3	17	0,01	1,5	68,67	3,73	0,13	1,42	6,87	0,05
A-Bulls Blood	52,16	20,75	0,01	0,56	41,5	7,63	0,08	25,25	7,8	0,06
H-Bulls Blood	49,23	16,67	0,02	1,07	74	9,53	0,10	5,77	5,73	0,06
A-Marino	56,70	17,25	0,02	1,05	50,25	4,83	0,13	4,33	7,7	0,12
H-Marino	50,49	16,33	0,02	1,81	64	4,63	0,17	1,6	6,2	0,10

The Cu content was slightly higher in aquaponic ‘Frillice’ and ‘Salanova Excite R2’ compared to hydroponic plants, while the opposite trend was observed for ‘Bulls Blood’. ‘Marino’ showed no difference in Cu content between treatments. The Fe content of all plant cultivars was higher in the aquaponic system, with one exception. The aquaponic ‘Bulls Blood’ had almost 50 % the content of hydroponic ‘Bulls Blood’. Hydroponic ‘Frillice’, ‘Salanova Excite R2’ and ‘Marino’ had a lower Mg concentration than aquaponic plants. ‘Bulls Blood’ was the only cultivar showing a lower Mg-content when grown in the aquaponic system compared to the hydroponic control. All aquaponic cultivars seemed to have a lower Mn content than hydroponic cultivars. The reverse was true when looking at Na content, with higher values found in all aquaponic cultivars when compared to hydroponic ones. Zn-content was higher for coriander than for the other cultivars regardless of treatment.

4.3.8. Nutrient content of fish

Table 4-9: The nutrient content of brown trout (Salmo trutta) and bleke (Salmo salar L.), based on fish harvested at the end of the comparative study.

Fish species	N g/kg	P g/kg	Total dry matter g/kg
Bleke	30,1	5,0	339,0
Brown trout	29,3	4,1	312,0

The nutrient analysis showed that bleke had a slightly higher N and P content compared to brown trout (Table 4-9). Bleke also had a higher total dry matter than brown trout.

4.3.9. Nutrient concentrations of the aquaponic system water, hydroponic control and aquaponic sludge

Table 4-10: Mean values of N, P, Cl and EC found in water samples from the aquaponic sump, analyzed during the 6 weeks of the comparative experiment. TAN-N = Total Ammonia Nitrogen.

Date	TAN-N mg/L	NO_2-N mg/L	NO_3-N mg/L	PO_4^- mg/L	Cl- mg/L	EC µS/cm
30.11.2015	0,121	0,089	109,0	5,40	97,4	1420
07.12.2015	0,088	0,064	98,0	4,90	92,2	1350
14.12.2015	0,068	0,076	96,0	4,20	82,7	1130
18.12.2015	0,104	0,084	98,5	3,74	76,6	1120
26.12.2015	0,106	0,068	97,2	3,52	74,0	1140
04.01.2016	0,088	0,072	111,0	2,62	68,8	1130
Average	0,091	0,073	100,140	3,796	78,860	1174,000

The nutrient concentration varied throughout the comparative study (Table 4-10). The amount of TAN-N varied from 0.068 – 0.121 mg/L. The nitrite varied from 0.064 – 0.089 mg/L and nitrate varied from 97.2 – 111.0 mg/L. The average value of nitrate-nitrogen was 100.140

mg/L. The phosphorous and chloride concentrations decreased steadily throughout the study. Electrical conductivity started out at 1420 but stabilized around 1130 – 1140 µS/cm.

Table 4-11: Average values of macro- and micronutrient concentration of aquaponic and hydroponic system water from water analysis done at NMBU.

Water source	TOT-N mg/L	Ca mg/L	Cu mg/L	Fe mg/L	K mg/L	Mg mg/L	Mn mg/L	Na mg/L	P mg/L	Zn mg/L
Aquaponics	89	140	0,0270	0,63	<0,51	4,4	0,00370	20,0	3,7	0,039
Hydroponics	64	130	0,0515	0,51	99,5	10,0	0,00705	5,1	19,0	0,195

There were differences in nutrient concentration between the aquaponic system water and the hydroponic control (Table 4-11). TOT-N and Ca concentrations were higher in aquaponic water than in hydroponic, while the K concentration was not detected in aquaponic water and 99.5 mg/L in the hydroponic water. The amount of Na was higher in aquaponic water compared to hydroponic, while the opposite was found for the concentration of P.

Table 4-12: Average values of TOT-N, P and suspended solids (SS) measured in aquaponic sludge.

Water source	TOT-N mg/L	P mg/L	SS mg/L
Aquaponic sludge	340	97	8700

The nutrient analysis of aquaponic sludge water (Table 4-12) resulted in a higher TOT-N value compared to the aquaponic system water (340 to 89 mg/L). The same was true for P with 97 mg/L compared to 3.7 mg/L in aquaponic system water. A suspended solid concentration of 8700 mg/L was also found.

5. Discussion

5.1. Plant growth and development of ‘Frillice’ in monoculture

The variation between the largest and smallest harvest weight of ‘Frillice’ could be explained by changes in temperature and sunlight from the onset of autumn or changes in nutrient production due to harvesting of rainbow trout. The later increase in yield toward the end of the study may point to a limited nutrient supply, although no visible nutrient deficiencies were observed. Reduction in growth due to aphids were limited during the monoculture experiment, and the later increase in yield suggest another reason behind the mid-September dip in yields. Weather station data from Landvik showed a gradual decrease in global radiation from August to October. The light intensity within the greenhouse therefore decreased despite using artificial lighting. The natural light intensity from the sun would add more natural light to the total light intensity within the greenhouse in August compared to September and early October because of the decline in sunlight intensity in autumn (Figure 0-1, Appendix C). The same decreasing trend was found when looking at the average temperature during the monoculture study. The average temperatures of 2015 were slightly higher than normal (Figure 0-2, Appendix C). Precipitation levels were found to be higher than normal during August and September, but lower in October (Figure 0-3, Appendix C). The much higher precipitation during September in addition to the lower radiation levels mentioned earlier could affect the growth of ‘Frillice’ negatively due to lower levels of photosynthetically active radiation (PAR). The radiation intensities were particularly low between 11.09. and 18.09., possibly explaining one reason behind the poor harvest registered at the 18.09.2015. A decrease in nutrient production due to the harvesting of mature rainbow trout could also explain the low harvest weights of mid-September.

The fresh weight per lettuce plant found in the literature study varied between 6 g – 327 g depending on source, cultivar and treatment (Table 2-6). Buzby et al. (2016) found Bibb ‘Rex’ lettuce harvest yields of 655.0 – 1343.8 g in low and high water flow, respectively, and 869.9 g when amended with fish waste. The fresh weight of plants varied between 21 – 45 g/plant with the highest weight resulting from the high water flow treatment. They also found Butterhead ‘Rhazes’ harvest yields of 189.7 – 161.4 g in low and high water flow, respectively, and 523.3 g when amended with fish waste, in an aquaponic system of 13 °C. The individual plant weights were ca. 6 – 17 g/plant with the highest weight resulting from the amended treatment. Skar et al. (2015) found ‘Frillice’ harvest yields between 9526 – 16482 g during the winter of 2015. The lettuce yields found by Buzby et al. (2016) were heavier cultivars that form loose heads, while the lettuce cultivars produced in the Landvik system were open leafed cultivars.

Both the largest and the smallest harvest yields of lettuce found in this study were higher compared to Skar et al. (2015) with the largest yield of 19540 g compared to 16482 g, and the smallest yield of 9820 g compared to 9526 g. This difference could be due to a difference in time of year between the studies. The current monoculture study was conducted from August – October while Skar et al. (2015) conducted their study from January – March 2015, resulting in higher natural light intensity in the first study compared to the latter. Increased radiation contributes to higher growth rates, thus increasing the potential yields obtained from production (Taiz & Zeiger, 2010).

The average individual plant weight of ‘Frillice’ was based on calculations and varied between 102.29 g in the smallest harvest and 203.55 g in the largest harvest. The individual weight from both harvests were higher than yields found by Sace & Fitzsimmons (2013) and Buzby (2016), while the largest harvest produced higher individual plant weights than what was found by Sikawa & Yakupitiyage (2010). The plant yield from the smallest harvest was below the findings of Sikawa & Yakupitiyage (2010), while both yields were lower than yields found by Pantanella et al. (2010) and Rakocy et al. (2011). Buzby (2016) and Sace & Fitzsimmons (2013) produced lettuce in a cold water system while the other studies were conducted in warm water systems, which may explain some of the differences in the observed yields because the temperature, radiation intensity and other factors varied between the studies.

All references except Buzby et al. (2016) and Skar et al. (2015) produced lettuce in warm water temperature systems. Lennard and Leonard (2004) found a green oak lettuce yield of 4.97 kg/m^2 and an individual fresh weight of 129.98 g/plant in a constant flow, NFT-system at a temperature of 22.0 °C. They also found a green oak lettuce yield of 4.47 kg/m^2 and an average individual plant fresh weight of 116.91 g in a DWC-system of similar temperature (Lennard and Leonard, 2006). ‘Simpson’ lettuce produced a yield of 173.73 g/m^2 in a DWC-system at 28.9 – 30.9 °C (Sikawa, D. C. and Yakupitiyage, A., 2010). ‘Integral’ yield varied between 2.37-2.71 kg/m^2 and an average fresh weight per plant of 118.6 – 135.3 g in the first trial with 50 % shade, and 5.67-5.70 kg/m^2 and an average fresh weight per plant of 283.3-285.2 g in the second trial in full sun. The system temperature was not mentioned (Pantanella, E. et al, 2010). This suggests that growing lettuce in 50 % shade will reduce the full sun yield by more than 50

%. Individual lettuce fresh weights of 265 g (green leaf ‘Nevada’), 269 g (red leaf ‘Sierra’), 314 g (romaine ‘Jericho’) and 327 g (romaine, ‘Parris Island’) were achieved in the UVI-system with an outdoor temperature of 25.1-27.5 °C (Rakocy, J. E. et al., 2011). The variation in lettuce yields found in the literature study shows that there are differences from one study to another, within systems of somewhat similar temperatures. All studies so far produced lettuce in monoculture.

5.2. Nutrient balance of ‘Frillice’ in monoculture

The nutrient concentration of aquaponic water varied between sampling dates, with the lowest concentration observed in September. The nutrient concentration of the water was closely linked to the feeding rate and amount of fish feed added to the system. A decrease in nutrient production resulted from the harvesting of mature rainbow trout and brown trout on September 14 2015. Replacing the fish with smaller bleke and brown trout could explain the low nutrient concentrations observed in mid-September, which could also explain the lower harvest yield. Restocking the fish disrupted the nutrient balance by reducing waste production as the fish biomass decreased. The reduced amount of fish waste lead to a subsequently lower nitrification activity of bacteria. The concentrations of all nutrients except P was lower in the water sample taken on the 25.09. compared to the other samples that were taken prior and later (Table 4-2). Lower nutrient content limits plant growth because the plant will have a lower assimilation rate of essential elements, subsequently leading to a slower growth rate (Taiz & Zeiger, 2010).

Variations in nutrient content between the replicates was observed even though they were grown in the same system, shared the same age and were otherwise treated equally. Each replicate was based on 24 ‘Frillice’ plants which should reduce genetic variation within each replicate. This suggests that the placement of the floating rafts could influence the nutritional content of the harvest. Nutrient assimilation increases with increasing temperatures and light intensity (Taiz & Zeiger, 2010). It is possible that the two replicates closest to the fish tanks could have received slightly higher light intensities from lights above the fish tanks, but this slight difference should be insignificant because of the low irradiance of the fish tank lights. Micronutrient content seemed to be more constant than macronutrient content. This could be because macronutrients are needed in higher concentrations, of which N and P are most essential to plant growth (Taiz and Zeiger, 2010). Rakocy et al. (2004b) found nutrient deficiencies in lettuce when the water in the UVI-system flowed from one 30.5 m hydroponic tank directly into another before returning to the sump and fish tank. This could be because plants take up nutrients as the water flows past their roots, reducing the nutrient concentration of the water so that plants further down the hydroponic tank take up lower amounts of essential elements. The Landvik system was only 5.0 m in length, but plants that were growing closer to the nutrient rich influent pipes could have access to a higher nutrient concentration than those closer to the effluent pipes. The difference between the influent and effluent might be insignificant. Nutrient deficiencies showing no visible sympotms can still occur without necessarily limiting plant growth. The continuous recirculation in DWC-systems could reduce the overall differences between plant placement within the hydroponic rafts, but this difference should be subject to further studies. Another explanation might be the arrangement of the artificial lights or subtle differences in sunlight due to a slight shading effect from a nearby house in the early morning hours.

5.3. Plant growth and development of lettuce, coriander and swiss chard in polyculture

5.3.1. Germination and root growth of seedlings

The second germination experiment showed a germination success between 60 – 100 % as shown in Figure 4-3. ‘Frillice’ had the highest germination success of 100 % even though the this germination experiment was dryer compared to the standard germination procedure used for ‘Frillice’ production at Landvik. The two other lettuce cultivars had a lower germination success of 82 %, while the swiss chard cultivars ‘Bulls Blood’ (86 %) and ‘Fordhook Giant’ (60 %) showed the highest intra-species variation. This suggests that genetic variation could be one factor explaining the difference in germination success. ‘Marino’ showed a germination success of 98 %, but was not compared to other coriander cultivars.

A few, straight roots growing out from the rockwool cubes were found to be the best characteristics when predicting the success of transferring seedlings from the germination area into the aquaponic and hydroponic systems. ‘Frillice’ showed root growth variation ranging from a few main roots to thinner roots resembling a spiders’ web. This variation was observed for all species and cultivars in the study and could be due to both genetic and environmental factors. The rockwool material of the sowing media provided support for the small seedlings while simultaneously forcing the plant roots to grow through it. Roots would grow downward through the rockwool cubes, just like any other germination media through gravitropism (a growth response to gravitational forces) and toward increased moisture levels. Plant roots are also able to grow around obstacles by responding to touch (thigmotropism) (Taiz and Zeiger, 2010), thus taking the path of least resistance through the growing media. Seedlings with thicker, fewer roots should penetrate the germination media in a shorter time than thinner roots. These were transferred first while seedlings with sub-optimal or poor root growth were transferred last. This process was unfortunately not random, meaning that the cultivars with the strongest roots were transferred into the hydroponic control first, and seedlings of subsequently poorer quality was transferred into the aquaponic system. This could lead to unintentional variation between systems. This variation was most evident for the swiss chard cultivar ‘Bulls Blood’, resulting in poor to non-existent seedling growth after being placed into the aquaponic system. The amount of ‘Bulls Blood’ plants were reduced from 24 to 12 during the experimental period due to plants not growing, wilting and dying.

5.3.2. Plant yield and growth

A weak trend suggesting that all cultivars except ‘Marino’ produced a higher yield and fresh weight per plant in the hydroponic control than in the aquaponic system was observed in Figure 4-4 and Figure 4-5. The plant density used in this comparative study was 33.6 and 36 plants/m^2 for the aquaponic system and the hydroponic control, respectively. This could explain the observations because a higher number of plants per square meter produces a higher yield under optimal growth conditions. Petrea et al. (2013a) observed that the plant yield decreased when the plant density increased ($p<0.05$), but this was not found in the current study. This could be due to a smaller difference between plant densities of the aquaponic system and hydroponic control compared to Petrea et al. (2013a). Increasing the plant densities might or might not show stronger trends of increased yield differences between the aquaponic and hydroponic systems until the plant density becomes too high and starts reducing yields.

This trend was not significant as ANOVA showed no difference between the average yield of aquaponic and hydroponic treatments ($p<0.05$). The difference in ‘Bulls Blood’ seedling quality between the aquaponic and hydroponic systems was not large enough to be of significance. This means that the aquaponic system produced plants that were similar to plants produced in the hydroponic system. 71.05 % of the variation in yield was explained by the General Linear Model.

Leaf weight and yield

Figure 4-5 showed possible weak trends of hydroponic ‘Frillice’ and ‘Salanova Excite R2’ obtaining a higher leaf fresh weight per plant than their aquaponic counterparts, and the opposite for hydroponic ‘Bulls Blood’ and ‘Marino’. Although the trends had no statistical significance, it is possible that the difference could be due to many factors. Two explanations could be the lower nutrient concentrations in the aquaponic water and a higher aphid occurrence within the aquaponic plant tanks compared to the hydroponic control. The lettuce cultivars had one plant per rockwool cube while ‘Marino’ and ‘Bulls Blood’ had multiple plants per cube. The poor quality of ‘Bulls Blood’ seedlings resulted in a high occurrence of wilting and plants dying. These plants were consequently removed from the rockwool cubes, reducing the number of plants per cube in the aquaponic system compared to the hydroponic control that showed no plant mortalities. This could have resulted in aquaponic seedlings having less shading and competition from within their cubes compared to hydroponic seedlings that had more plants in their cubes, and possibly explaining the trend of higher fresh weight per plant being observed in aquaponic ‘Bulls Blood’. The lower plant density could explain the same trend in aquaponic ‘Marino’ as the plants would grow in less shade and competition.

ANOVA showed that the yields of ‘Frillice’ and ‘Salanova Excite R2’ were significantly higher than those of ‘Marino’ and ‘Bulls Blood’ ($p<0.05$). No significant difference was shown between ‘Frillice’ and ‘Salanova Excite R2’ nor between ‘Marino’ and ‘Bulls Blood’. ‘Frillice’ obtained an aquaponic yield of 2570.88 g/m^2 and a hydroponic yield of 3038.32 g/m^2, respectively. The average fresh weight of both aquaponic and hydroponic ‘Frillice’ was 80.46 g/plant. The average yield and fresh weight of ‘Frillice’ grown in the earlier, preliminary monoculture study varied between 3436.94 – 6839.28 g/m^2 and 102.29 – 203.55 g/plant in the largest harvest. The largest harvest provided ‘Frillice’ plants with a weight almost twice the smallest harvest. The ‘Frillice’ plants grown in a monoculture during the comparative study obtained an average fresh weight 134.16 g/plant (n=3), showing that the yield and individual weight of ‘Frillice’ grown in polyculture with coriander and swiss chard were lower both compared to the preliminary monoculture from the first study and the monoculture grown during the comparative study. It is important to note that the preliminary monoculture and the monoculture grown simultaneously with the comparative study were two different experiments. Both monocultures were grown in a staggered production while ‘Frillice’ in the polyculture study was grown in a batch production. This means that the polyculture ‘Frillice’ in the comparative study had two additional weeks of growth time compared to the two monocultures and still produced poorer results. The plants were completely randomized within each replicate in both treatments, with lettuce growing in close proximity to taller coriander and swiss chard cultivars. A shading effect from taller plants could explain the lower yield, fresh weight and height of ‘Frillice’ grown within the polyculture. The same could be true for ‘Salanova Excite R2’ due to belonging to the same species.

‘Salanova Excite R2’ plants produced an aquaponic yield of 3004 g/m^2 and a hydroponic yield of 3282.5 g/m^2. The average fresh weight of ‘Salanova Excite R2’ plants was found to be 89.00 and 91.18 g/plant in aquaponic and hydroponic systems, respectively. The fresh weight of lettuce leaves from the comparative study were comparable to lettuce fresh weights of 73.1 – 78.5 g/plant achieved by Sace & Fitzsimmons (2013). The temperature of the two systems was rather similar (16 °C in this study and 16.3 – 24.0 °C reported by Sace & Fitszimmons, 2013). The lettuce yields achieved in this study were higher than that of Sace & Fitzsimmons (2013) and Buzby (2016), but lower than all other lettuce yields shown in Table 2-6 of the literature study. Pantanella et al. (2010) showed large differences between lettuce plants grown in 50 % shade and full sun, with the shade experiment producing a yield less than 50 % of the full sun experiment. The yield of ‘Salanova Excite R2’ was lower than the lettuce yields in the shade trial by Pantanella et al. This could be explained by a difference in temperature, other cultivars, light intensity or other factors that were different between the studies. Pantanella et al. did not record their system temperature.

Polyculture results cannot easily be compared to monoculture results from literature. It would therefore be more accurate to compare the fresh weights of ‘Frillice’ grown in a monoculture during the comparative study (136.16 g/plant) with monoculture results found in literature. This would result in a monoculture fresh weight that was barely higher than the fresh weight of lettuce produced in 50 % shade as reported by Pantanella et al. (2010). ‘Salanova Excite R2’ and ‘Frillice’ were shown to have a significantly higher leaf weight than ‘Marino’ and ‘Bulls Blood’ ($p<0.05$), but no significant difference was found between the leaf weights of ‘Frillice’ and ‘Salanova Excite R2’ nor between ’Marino’ and ‘Bulls Blood’ ($p<0.05$). 71.05 % of the variation in leaf weight was explained by the General Linear Model. This difference is explained mostly by genetic differences between species, as lettuce weighs more than either of the other two cultivars.

The aquaponic fresh weight of ‘Marino’ obtained in the current study was within the range of 0.2 g – 35 g/plant found in literature (Silva et al., 2015; Buzby et al., 2016). Coriander plants seemed to obtain the highest yield when grown in a low water flow treatment compared to high flow and amended treatments, but there was no statistical difference to support this observation (Buzby et al., 2016). Increased nitrogen fertilization and water availability produced higher yields until a nitrogen level of 90 kg/ha was reached (Lenardis et al., 1999). These findings suggest that moderate nitrogen fertilization may produce higher coriander yields than high nitrogen fertilization. This observation is supported by the lower nitrogen concentrations of the Modified Sonneveld's solution used for hydroponic herb production, compared to the UA CEAC Recipe and the Modified Hoagland solution used for fruiting vegetables that require higher nitrogen concentrations. Plants grown by Buzby et al. were grown in 13 ° C making their results comparable to the comparative study. Both the aquaponic and hydroponic yields of 'Marino' were higher compared to Silva et al. (2015), but lower compared to Buzby et al. (2016).

The coriander yields should be less affected by shading because 'Marino' produced the tallest leaves. Only large swiss chard plants could influence the coriander growth because of their similarly tall leaves. Coriander plants in the hydroponic system could have been more affected by shading due to a higher planting density compared to the aquaponic system. Additional shading effects could occur because of multiple plants being grown in the rockwool cubes and competing for light and nutrients within the rockwool cube. This resulted in one or two plants dominating the rockwool cube while the rest of the plants would grow much slower. The fresh weight per plant could possibly increase if only one or two plants were grown in each rockwool cube.

Shading became a possible growth limiting factor that also affected the swiss chard plants in the aquaponic system. The problem increased when the other cultivars grew faster than 'Bulls Blood' plants, creating a shading effect on the smaller 'Bulls Blood'. This was less apparent in the hydroponic control due to stronger, healthier plants resulting from a higher seedling quality. A later shading effect from mature 'Bulls Blood' plants was noticed in the hydroponic control because the (not significantly) taller plants started shading the other cultivars. The lower survival rate of 'Bulls Blood' grown in the aquaponic system was mainly explained by the lower seedling quality and poor root growth observed from the start of the comparative study. Roots that were too short to reach into the aquaponic water resulted in wilting and eventual plant death.

Individual plant leaf weights of 'Bulls Blood' found in the current study were higher than those reported by Buzby et al. (2016), stating that 'Bulls Blood' varied between ca. 0.7 – 2 g/plant, primarily depending on nutrient availability, while other swiss chard cultivar yields varied between ca. 1.20 – 1.5 g/plant and ca. 1.6 –3.5 g/plant. Both ‘Bulls Blood’ and ‘Early Wonder T. T.’ produced significantly higher individual yields when grown in the amended treatment ($p<0.07$). One suggested reason behind the poor yields was that autumn influenced the yield, and that growing swiss chard in spring resulted in normal growth (Buzby et al., 2016). Buzby et al. (2016) conducted their experiments in a flow-through aquaculture system with no water recirculation. This would influence the growth of swiss chard negatively, as their system water would have a lower nutrient concentration compared to a recirculating system where nutrient concentrations accumulate over time. The minimum temperature needed for swiss chard germination is 4 – 8 °C (Whiting et al., 2014; LOG). Swiss chard can tolerate brief periods of frost, but thrive in temperatures from 4-10 to 23.9 °C (Whiting et al., 2014; Drost, 2010). Higher temperatures will result in lower growth and decreased quality (Drost, 2010). This means that the low ‘Bulls Blood’ yields observed in this study and in literature could explained by a lower nutrient concentration, genetic suitability to hydroponic/aquaponic production or other factors.

Total fresh weight of leaves per cube

The growth parameter leaf weight per cube was measured because the four cultivars had a different number of plants per rockwool cube. Both lettuce cultivars had only one plant per cube, while ‘Marino’ and ‘Bulls Blood’ had multiple. This is the reason for the identical results found for leaf weight per plant and leaf weight per cube for the two lettuce cultivars in Table 4-3 and the reason why the leaf weight per cube of ‘Salanova Excite R2’ and ‘Frillice’ was significantly higher than the leaf weight per cube of ‘Bulls Blood’ and ‘Marino’ ($p<0.05$). The individual leaf weights of ‘Marino’ and ‘Bulls Blood’ plants showed a large variation within each rockwool cube. One explanation could be shade avoidance responses between multiple plants grown in one rockwool cube. The trend observed in ‘Marino’ was similar in ‘Bulls Blood’, with one or two plants dominating the growth while the others grew slower. Reducing the number of plants per cube might or might not increase the fresh weight per cube by reducing shading from other plants. A possible trend in Figure 4-5 showed that plants grown in the hydroponic control resulted in higher leaf weights per cube for all cultivars except ‘Marino’. The trend was weakest for the two lettuce cultivars, but stronger for ‘Bulls Blood’. This trend could be explained by a higher number of plants per cube within the hydroponic control due to a higher mortality rate in aquaponic ‘Bulls Blood’ seedlings resulting from the low root growth.

ANOVA showed no statistical evidence to support the observed trend, making the trend non-significant.

Total fresh weight of roots per cube

There was a small, but observable difference between the aquaponic and hydroponic root weights of the two lettuce cultivars in this study, with the hydroponic control resulting in the heavier root weights (Figure 4-5). ‘Bulls Blood’ showed a stronger trend of obtaining heavier root weight when grown in the hydroponic control. Aquaponic ‘Marino’ produced a slightly higher root weight than the hydroponic ‘Marino’. The root weight of ‘Bulls Blood’ and ‘Marino’ could be higher than what was found in this study because of roots breaking and being damaged especially during the later weeks of the data collection period. The lower volume of the hydroponic control may have led to increased root entanglement compared to the higher volume of the aquaponic system. ANOVA showed no statistical evidence to support this trend ($p<0.05$). The total fresh weight of roots per cube varied significantly between cultivars, with ‘Marino’ roots being significantly heavier than all other cultivars ($p<0.05$). 43.86 % of the variation in root weight was explained by the General Linear Model. This percentage is lower than what was observed for the fresh weight of leaves, meaning that there is less evidence to support the observed results. This could be a result of the low number of plants used in the comparative study. An increase in plants per cultivar from 6 plants per replicate in the aquaponic system and 3 plants per replicate in the hydroponic system might or might not increase this percentage. The fresh weights of ‘Marino’ roots were 16.75 and 15.40 g/cube in the aquaponic system and the hydroponic control, respectively. Since all plant parts are used as spice in Asian cuisines, fresh weight of roots could also be considered part of the total yield of coriander (Verma, A. et al., 2011), reducing the waste production of the aquaponic system. This would have to be specified as root yield to avoid even more confusion between coriander plant parts (the plant, seeds and roots). The root weight would naturally differ between plant species such as lettuce, coriander and swiss chard due to biological factors. The higher root weight of coriander might be due to a biological need of more support due to its relatively taller size.

5.3.3. Leaf count development

No statistical difference was found between the average leaf count of aquaponic and hydroponic treatments ($p<0.05$). The leaf number development of both aquaponic and hydroponic plant species and cultivars start at around the same leaf number, and seem to follow similar growth patterns. The leaf development of each cultivar was expected to be similar due to having similar genes, but nutrients were suspected to affect the development rate. Significant differences were found between the average leaf count and plant cultivars ($p<0.05$). 'Salanova Excite R2' had the significantly highest number of leaves out of all the studied cultivars with a leaf count of 23.19 ($p<0.05$). This means that 'Salanova Excite R2' developed the highest number of leaves during the study. 'Frillice' had a statistically higher number of leaves than 'Bulls Blood' and 'Marino', but ANOVA showed no statistically significant difference between the average leaf number of 'Bulls Blood' and 'Marino' (Figure 4-6, Table 4-4). The similar leaf count of the two lettuce cultivars and the much lower leaf count of 'Bulls Blood' and 'Marino' suggest that the differences are due to the cultivars belonging to three different species. One biological difference would be the bushy growth pattern of the lettuce compared to the more vertical leaf growth of 'Bulls Blood' and 'Marino'.

'Frillice' grown in monoculture during the comparative study obtained a final leaf count of 24.44, which is much higher than the 18.70 obtained by 'Frillice' grown in the comparative study. This shows that 'Frillice' can produce a higher number of leaves when grown in a monoculture, a fact that might be shared by 'Salanova Excite R2', 'Bulls Blood' and 'Marino'. 'Bulls Blood' could theoretically have produced more leaves during the experimental period if the transfer into aquaponic and hydroponic systems had been quicker and more successful. The later transfer date resulted in a shorter growth period and a potentially lower final leaf number by the end of the experiment. 'Marino' was harvested one week earlier than the other cultivars due to a suspected nutrient deficiency of K or disease turning leaf petioles and new shoots into black, curled up leaves that eventually fell apart as if the shoots had rotted. 'Marino' could potentially have produced more leaves if left in the system for one week longer under optimal conditions. It seemed like 'Salanova Excite R2' produced more leaves at an increasing rate compared to the other cultivars in the last week of the experiment. This could be a result of less shade and more light intensity in the absence of 'Marino'.

5.3.4. Leaf height development

ANOVA showed no statistical difference between the average maximum leaf height of aquaponic and hydroponic treatments ($p<0.05$). ‘Marino’ and ‘Bulls Blood’ obtained significantly longer leaves than both ‘Salanova Excite R2’ and ‘Frillice’. ‘Marino’ produced the longest average leaves at 31.02 cm. There was no significant difference in leaf length between Salanova Excite R2 and Frillice ($p<0.05$) (Figure 4-7,Table 4-5). Silva et al. (2015) reported a local commercial coriander harvest height 10-15 cm, which means that coriander plants could be harvested after only 2-3 weeks of growth. The harvest date of coriander varies with what plant part is harvested as plants used for baby leaf salads can be harvested much earlier than mature plants and coriander seeds.

‘Frillice’ grown in monoculture during the comparative study once again seemed to produce higher leaves (17.42 cm) when compared to the 14.30 cm achieved by ‘Frillice’ grown in the polyculture of the comparative study. No statistical analysis was done on this difference to prove that it was significant. The growth rate of leaf height of all cultivars seems to slow down after 4 weeks of growth in the hydroponic control. This slower growth rate was observed to be one week later in the aquaponic system. Aquaponic ‘Marino’ was an exception, showing no signs of reduced growth rate at the time of harvest (Figure 4-7). This could be explained by a natural slow down in growth rate as the optimal harvesting date passed, or that the nutrient solution in the hydroponic control became more unbalanced. The slight increase in leaf height of all remaining cultivars observed from the second last to the last measurement date is most probably due to increased light intensities and less competition for nutrients in the absence of ‘Marino’. ‘Bulls Blood’ seemed to have a higher increase in the hydroponic control than in the aquaponic system, probably due to the smaller volume and the higher competition expected in the control. A weak, opposite trend was observed for the increase in leaf height of ‘Frillice’ and ‘Salanova Excite R2’.

5.3.5. Root length development

No statistical difference was found between the average maximum root length of aquaponic and hydroponic treatments ($p<0.05$). ‘Bulls Blood’ and ‘Marino’ obtained the longest roots at the time of harvest ($p<0.05$) with only 18.12 % of the variation in root length being explained by the General Linear Model. There was a high degree of variation within each replicate, especially in the hydroponic control because roots of different cultivars and individuals grew into each other, resulting in mechanical damage such as cutting of the main roots while trying to untangle them. This may explain the apparent slower growth rate of root length observed in all cultivars except 'Bulls Blood' after three weeks of growth in the hydroponic control. Plants grown in the aquaponic system seemed to have a slower root growth after five weeks, except for 'Marino' that seemed to slow down after two weeks of growth. The later slowdown in root growth of 'Bulls Blood' could be explained by genetic factors that kept root growth almost constant in the hydroponic system. The longer root growth could also be a response to higher competition pressure or more optimal nutrient concentrations within the hydroponic control water compared to the aquaponic system. The early harvest of 'Marino' increased the light intensities and lowered nutrient competition. This might have contributed to a longer root growth period as a relative increase in nutrient availability could provide a higher nutrient assimilation rate, counteracting a decrease in root growth that would have occurred otherwise. The actual lengths of all cultivar roots would have been even longer if they had not broken off and become entangled in each other between measuring dates.

5.3.6. Plant production in the presence of aphids

The presence of aphids could be a possible reason for the lower total yield and fresh weight of lettuce compared to the 'Frillice' produced in monoculture during the comparative study. The extent of the infestation was so severe that whole lettuce harvests had to be discarded because the leaves collapsed. The pest problem started while the plants used in the comparative study were at the seedling stage. Aphids were observed to be more abundant on 'Frillice' plants than 'Salanova Excite R2', probably because the aphids had attacked the monoculture of 'Frillice' first. Aphids became more numerous on 'Salanova Excite R2' as time went on, suggesting that aphids left 'Frillice' due to a crowding effect from the near exponential population growth. They migrated into the polyculture containing the test plants for the comparative study and seemed to prefer 'Salanova Excite R2' plants. Aphids are known to be polyphagous pests, feeding on host plants from many different families. The green peach aphid (*Myzus persicae*) has been recorded to have host plants in more than 50 families (Schoonhoven et al., 2005). 'Marino' and 'Bulls Blood' were less affected than the lettuce plants, with the lowest aphid numbers being observed on 'Bulls Blood'. This suggests that 'Bulls Blood' was a host plant with a higher degree of resistance to the aphid species in question. It could also be that the presence of more desired host plants shielded 'Bulls Blood' from taking excessive crop damage as the pests preferred lettuce plants. Pests were initially controlled by removing or killing them manually while awaiting the arrival of biological agents and this may have slowed down what could otherwise have resulted in crop failure, necessitating a total remake of the comparative study. The late introduction of biological agents including parasitic wasps and predatory mites resulted in whole monoculture harvests of lettuce being lost because they were not able to control the pests sufficiently. The biologic agents became more effective as they increased in numbers until they were providing enough control to keep the pests from destroying the plant crops. The early damage could nonetheless affect the later growth rate of the plants, possibly explaining the lower yields of the aquaponic lettuce cultivars. Dedryver et al. (2010) reported that wheat plants that were attacked by aphids early in their development resulted in lower grain weights. This could be true for other crops as well, providing one possible explanation of the lower yields observed in ‘Frillice’ grown in polyculture compared to the final harvest weight of ‘Frillice’ grown in monoculture during the comparative study. Reduced growth due to pest damage would be much more prevalent in ‘Frillice’ and ‘Salanova Excite R2’ than in ‘Marino’ and ‘Bulls Blood’.

5.3.7. Fish yield and size

The average fresh weights and sizes were 122.37 g, 22.07 cm and 177.67 g, 23.41 cm for bleke and trout, respectively. Bleke showed a lower variation in weight when compared to trout. The highest individual weight and size observed during the experimental period was 205.3 g and 490.4 g for bleke and trout, respectively. The specific growth rate of bleke showed a greater variation between the two fish tanks as well as a lower average value than trout. The highest specific growth rates were 0.22 and 0.37 for bleke and trout, respectively. This means that trout grew almost twice as fast as bleke. This was naturally reflected in the higher harvest weights of trout, while being only slightly longer in size than bleke. The very low specific growth rate of 0.08 found in fish tank 2, suggests that something affected the feeding and weight gain of bleke so much that the fish barely gained weight. These results show that trout would perform better as a fish crop for a cold water aquaponic system. This seems logical because trout has been bred specifically to aquaculture production while bleke only has been bred to restock natural populations of the species (Homme, J. M. Personal communication, October 2015). The difference in specific growth rates between the two fish species could be explained by this discrepancy too. Bleke showed observably more stressed behaviour during the study compared to trout, resulting in sub-optimal feeding behavior. McCormick et al. (1998) found that the growth rate of Atlantic salmon (*Salmo salar*) parr, a fish closely related to bleke, was 34 and 50 % lower when stressed once and twice a day over a 30-day period, compared to a nonstressed control treatment. The observed lower feeding rate of bleke during the experimental period of both studies lead to fish feed reductions to minimize the amount of uneaten feed in the swirl filters of the aquaponic system. Stress tolerance may have been one major factor explaining the difference in harvest weights, as stressed fish perform much worse than healthy fish in aquaculture. Bleke fish also showed high mortality rates during the monoculture study of ‘Frillice’. One possible reason for this is that it might not tolerate the nitrate levels found in the aquaponic water, although stress was expected to be the main reason for the higher mortality rates.

5.4. Nutrient balance of lettuce, coriander and swiss chard in polyculture

5.4.1. Nutrient content of plants

The average nutrient contents of ‘Frillice’ produced in the comparative study were similar to what was found in the preliminary monoculture study. The ‘Frillice’ contents of N, Cu, Fe, K, Mg, Mn and P were higher in the comparative study when compared to the monoculture study. ‘Frillice’ contents of Ca, Na, Zn were lower in the comparative study than in the monoculture study. These differences are similar in ‘Salanova Excite R2’. There were three main differences between the comparative and monoculture studies. Chelated iron was added during the comparative and not during the monoculture study. Larger sized rainbow and brown trout were replaced by younger bleke and brown trout fish, reducing nutrient production, and the comparative study was conducted under lower radiation levels due to the time of year. These factors may have affected the observed differences.

The literature review could not provide sufficient values of the nutrient content of coriander or swiss chard to provide comparisons with the current study. The nutrient comparisons was therefore mainly based on the nutrient content of lettuce cultivars. The nutritional content of N, Ca, Fe, K, Mn and Zn found in all plant cultivars in this study were higher than what was reported by Sikawa & Yakupitiyage (2010). The Cu content of aquaponic lettuce (0.02 g/kg) in the current study was similar to what was found in their unfiltered DWC treatment (0.022 mg/g). Overall Cu content of the current study was lower than their unfiltered means and close to 50 % of the Cu content found in their filtered means as reported by Sikawa & Yakupitiyage (2010). Unfiltered means were based on nutrient content of plants irrigated with unfiltered pond water, while filtered means were based on filtered pond water. ‘Frillice’ and ‘Salanova Excite R2’ nutrient content of N, Ca, Fe and Na were higher, but K and Mg concentrations were lower when compared to findings reported by Pantanella et al. (2010). Pantanella et al. reported a P content that was higher in their first crops, similar in their low density treatment and lower than the high density treatment of their second crop compared to the current comparative study.

The N, P, K contents of aquaponic ‘Frillice’ and ‘Salanova Excite R2’ obtained in the comparative study were lower than those reported by Al-Hafedh et al. (2008), who found that a fish feed to plant growth area of 56 g/m^2/d resulted in an aquaponic lettuce nutrient content of 172.7, 13.3 and 64.1 mg/L for N, P and K, respectively. The N, P, K contents of hydroponic ‘Frillice’ and ‘Salanova Excite R2’ of this study were much lower than the hydroponic lettuce content of 2800, 190 and 1500 mg/L for N, P and K reported by Al-Hafedh et al. (2008), respectively. The precise feeding rate used in the current study is unknown, but presumed to be similar to the 36 g/m^2/d used in Skar et al. (2015) because both studies were conducted in the same aquaponic facility with the mostly similar fish. The feeding rate reported by Skar et al. (2015) is lower than the one used by Al-Hafedh et al. (2008). A lower fish feeding rate will result in a lower nutrient concentration in the system water and a subsequently lower nutrient content within plant tissue (Buzby et al., 2016), possibly explaining some of the differences noted between the current study and Al-Hafedh et al (2008). Al-Hafedh et al. (2008) reported a water temperature of 25.5-29.6 °C, which contributes to a faster nutrient assimilation and growth rates compared to the lower temperature of 16 °C in the current study. There are most probably genetic differences between the three plant species that influence their nutrient uptake and nutrient content, although these values were quite similar across plant cultivars. The main differences in terms of nutrient content was determined by the aquaponic and hydroponic systems, not plant cultivars. One notable difference was that the plants grown in the aquaponic system contained a much higher amount of Na than their hydroponic counterparts. Aquaponic ‘Bulls Blood’ contained the highest amount of Na of all cultivars. High Na content could interfere with the nutrient uptake of other nutrients such as K, Ca and Mg. Reduced Ca due to high Na content can result in tip-burn symptoms that reduce the quality of marketable lettuce drastically (Rakocy et al., 2006).

5.4.2. Nutrient content of fish

The nutrient contents of N and P found in bleke and brown trout were similar. Bleke contained slightly higher levels of N and P than brown trout. The same was true with total dry matter content. These small differences could be explained by genetic factors as the environmental factors were kept identical for both fish species with one exception; bleke fish were fed less often than brown trout. Differences in feeding rate as well as exploitation rate of nutrients in the fish feed have been found to vary between fish species. Norges Forskningsråd (2006) reported that trout consumed higher amounts of fish feed when compared to salmon and Atlantic cod (*Gadus morhua*). The Atlantic cod compensated this difference by having a 30 – 40 % higher feed utilization rate than the other fish species. There are indications of salmon having a 20 – 30 % higher feed utilization rate than trout (Norges Forskningsråd, 2006). The fact that bleke is closely related to Atlantic salmon means that the higher nutrient content found in bleke could be explained by similar genetic factors resulting in a higher feed utilization rate although they were fed less frequently than brown trout.

5.4.3. Nutrient concentrations of aquaponic water

The pH value determines which if nitrogen is most abundant as ammonium and ammonia. A pH below 7.0 leads to a higher relative concentration of ammonium while a pH above 7.0 leads to a higher relative concentration of ammonia. Increasing concentrations of ammonium is beneficial for plant growth while ammonia can poison or kill fish at very low concentrations (Tyson, 2007). The Landvik system is operating at a pH-value of 6.74 (Table 3-2), which is a result of a compromise between the optimal pH of fish (6.5 – 8.5), nitrifying bacteria (7.5 – 9.0) and plants (5.5 – 6.5) (Tyson, 2007; Rakocy et al., 2006). Both pH and temperature affects nutrient concentrations of the aquaponic water by promoting or inhibiting nitrification and nutrient availability. While the pH determines the plant availability of many essential elements, the temperature either helps increase or decrease the nutrient assimilation rate of plants. Decreasing temperatures can lead to lower nutrient assimilation rates that could lead to nutrients accumulating within the aquaponic water, disrupting its nutrient balance. Keeping a close watch on both pH and temperature and keeping the values within optimum ranges could therefore mean the difference between a well-balanced, healthy aquaponic system and total system collapse.

The weekly measurements of water quality parameters (Table 4-10) show variations in concentrations from one measuring date to another. Nitrite levels increase and decrease with the levels of total ammonia nitrogen, just like the nitrate levels increase and decrease with the nitrite levels. This is an effect of bacterial assimilation of each nitrogen form. The concentrations of P and Cl decreased during the experimental period while the EC stabilized at levels that were lower than the hydroponic control water and the 1700 µS/cm reported by Skar et al. (2015). These values were measured in the same aquaponic system, with only one significant change between the two studies; the fish in the current study were fed less due to their smaller size when compared to the larger rainbow trout and brown trout used in the experiment conducted by Skar et al. (2015). An average EC of 500 µS/cm was measured in the UVI-system (Rakocy et al., 2004b) producing basil and lettuce, while Morgan (2016) recommended an EC of 1600 – 1800 µS/cm for sweet basil and cilantro, values that are both much lower and higher than what was observed in the current study. The EC levels of the comparative study were also higher than the average EC levels of 541.16 (lettuce) and 649.04 µS/cm (spinach) reported by Blidariu et al. (2013) and Petrea et al. (2013), respectively.

TAN-N, NO_2-N and NO_3-N concentrations reported by Sikawa & Yakupitiyage (2010) varied between 2.22 – 2.67, 0.01 – 0.17 and 1.54 – 4.02 mg/L, respectively. Their study showed relatively high values of total ammonia nitrogen due to the use of filtered and unfiltered catfish pond water as irrigation water for lettuce. This resulted in a shorter nitrification time, explaining the higher ammonia levels. The ammonia levels in the current study were lower than reported by Sikawa & Yakupitiyage due to a higher nitrification rate in the Landvik facility. Sikawa & Yakupitiyage reported similar nitrite levels as the current study, while their nitrate values were lower. Buzby et al. (2016) reported higher ammonia and nitrite concentrations than what was measured in the current study, while their nitrate concentration of 0.19 mg/L was far below the average value of 100.14 mg/L found in this study. One reason for this result is that this comparative study used a recirculating system while Buzby et al. (2016) used a flow through system with no water recirculation. The higher nitrate concentrations present in the current study should produce higher yields than the nitrate concentrations found in the other studies because increased nitrate results in increased plant growth.

The average values of TAN-N and nitrite were both within the requirements of all aquatic species shown in Table 2-5. The average value of nitrate was higher than what most fish could tolerate, thus excluding aquaponic production of arctic char, Atlantic salmon smolt, pike perch and sturgeon, all of which have nitrate tolerance levels below 100 mg/L. European eel could potentially be produced as it tolerates nitrate levels up to 100 mg/L, but a slight increase in nitrate would most probably result in crop failure or toxicities. Rainbow trout is better suited to a cold water aquaponic facility than other aquatic organisms shown in Table 2-5. This is because rainbow trout tolerates all nutrient concentrations measured during the current study. European lobster might not be suited for production judging by the low TAN-tolerance level unless the aquaponic system has an optimal nutrient balance with a very low concentration of TAN. Brown trout (Skar et al. 2015) and freshwater prawns (*Macrobrachium rosenbergii*) (Sace & Fitzsimmons, 2013) have also been shown to thrive in cold water aquaponic systems.

Water samples from the aquaponic system showed higher nutrient concentrations of N, Ca, Fe and Na when compared to the hydroponic control. The concentrations of Na could become toxic to both fish and plants if allowed to accumulate to 50 mg/L, which was twice the measured values (Rakocy, 2006). This means that the Na concentration was well below levels of concern. Aquaponic water was also found to have lower concentrations of Cu, K, Mg, Mn, P and Zn than the hydroponic control. Different nutrient concentrations between the two treatments could result in the small, non-significant differences in yield and fresh weight observed between aquaponic and hydroponic plants. K concentrations were too low to be detected, thus explaining the lower K content of aquaponic cultivars found in section 4.3.7. K deficiency is known to increase dark respiration, decreases plant growth while also reducing transport and synthesis of photosynthates (Havlin et al., 2005). Almost all of the nutrient concentrations found in the aquaponic system were lower than recommended. N, Fe, K, Mg, Mn, P and Zn concentrations were all lower compared to recommended values from nutrient solutions used for hydroponic plant production (Table 2-3). Ca concentrations were higher than Jack's HydroFeED and the Modified Sonneveld's solution, similar to Jack's Hydroponic (5-12-26) + Calcium nitrate, and lower than both the UA CEAC Recipe and the Modified Hoagland solution. This meant that the Ca concentrations of the aquaponic water were higher than what was recommended for lettuce and herbs, similar to recommendations for leafy vegetables such as lettuce, coriander and swiss chard, and lower than hydroponic solutions used mainly for fruiting vegetables such as tomatoes. The Cu concentration of the aquaponic system water was higher than the Modified Sonneveld's solution, similar to the Modified Hoagland solution and lower than the other hydroponic solutions shown in Table 2-3. The concentration of the aquaponic water was therefore within or above recommended values for the production of leafy vegetables. Na concentrations were not mentioned in the table because it is added with other essential elements.

Most of the nutrient concentrations in the hydroponic control were also found to be lower than recommended. N, Fe, K, Mg, Mn and P concentrations were all lower in the hydroponic water of this study compared to recommendations of standard hydroponic nutrient solutions (Table 2-3). The Ca concentration was similar in the hydroponic and aquaponic water, while the Cu concentration was lower than recommended for lettuce and herbs, but higher than or similar to the other hydroponic solutions (Modified Sonneveld's solution, UA CEAC Recipe, Modified Hoagland solution). The hydroponic concentrations of K and Mg, although lower, were close to the recommendations for lettuce.

The aquaponic nutrient concentrations found in the current study were higher than the concentrations of N and P, but lower than K reported by Al-Hafedh et al. (2008). They found that a fish feed to plant growth area of 56 g/m^2/d resulted in an aquaponic lettuce nutrient concentration of 2.3, 3.6 and 19.0 mg/L for N, P and K, respectively. They used the Hoagland solution as their control, with a nutrient concentration of 210, 31 and 234 mg/L of N, P and K, respectively. This nutrient solution contained higher nutrient concentrations than the hydroponic control of the current study. Rakocy et al. (2004b) found that all aquaponic water quality parameters except the concentration of Zn, Cu and Fe were substantially lower than hydroponic recommendations when producing okra in the UVI-system. The lower nutrient concentrations found in aquaponic literature still produced plant yields comparable to hydroponic systems because of continuous nutrient production resulting in a more stabile nutrient assimilation rate, requiring only small amounts of nutrient supplementation.

The TOT-N and P concentrations of aquaponic sludge shown in Table 4-12 are much higher than what was found in the aquaponic system water or within plant cultivars. The solubility of P decreases significantly in pH-values beneath 5.5 or above 7.5 (Taiz, L. and Zeiger, E., 2010). The pH of the aquaponic water was 6.74 on average during the preliminary monoculture study, and remained close to this in the comparative study. This pH should keep P and other nutrients should soluble within the aquaponic water. The high N and P concentration of aquaponic sludge is likely to be due to the sludge containing a mixture of highly concentrated uneaten fish feed and fish feces that contain varying amounts of P. An aquaponic system operating at a higher pH would see an increased precipitation rate of P. The pH of the Landvik system is slightly below the optimal range for nitrification (7.0-9.0) reported by Rakocy et al. (2006) resulting in a lower amount of available nitrate. The high concentration of suspended solids suggest that the sludge is rich in other elements as well.

5.5. Thoughts on improving the research quality of this and similar studies

The choice of randomizing the placement of the four plant cultivars within each replicate may have limited the yield, fresh weights and leaf height of all plant cultivars primarily due to shading effects. An experimental setup where each cultivar is produced in a monoculture could potentially result in higher values of all mentioned growth parameters compared to what was achieved in this comparative study. The only exception to this would be leaf count as it would stay relatively constant regardless of cultivation method. It is evident that the yields and leaf height of 'Frillice' were lower in 'Frillice' produced within the randomized polyculture than 'Frillice' produced in a monoculture during the comparative study. This result is expected to be similar for 'Salanova Excite R2' because both cultivars belong to the same species. This same trend could be true for 'Marino' and 'Bulls Blood' as well. Instead of placing 6 randomized plants of 4 cultivars into the same plant tray containing 24 holes, one plant tray should be filled with 24 plants of the same cultivar thus creating a monoculture. Each tray could then be treated as one quarter of a replicate, but this would require more space than what was available during this study. This would eliminate shading from taller plant cultivars and species and result in yields that are easily transferable to commercial production.

Limiting the number of 'Marino' and 'Bulls Blood' plants per rockwool cube to 1-2 would most probably result in higher yields per plant because the shade avoidance responses would be lower and plants would grow better than what was observed in this comparative study.

The importance of finding cultivars with similar or short germination and root growth times proved very important in the beginning of this study. The time of seedling transfer was delayed due to the slower root growth and development of 'Bulls Blood'. The original germination procedure developed for 'Frillice' did not result in satisfactory root growth of the selected cultivars. The slow root development was improved when the germination procedure was changed so that the rockwool cubes became drier than originally planned. 'Bulls Blood' may require even drier cubes or other factors to stimulate sufficient root growth in the same time as the other cultivars.

The importance of biological control cannot be overestimated. Successful pest and disease control depends on minimizing the time from pest or disease discovery to introduction of biological agents. Aphids and other pests enjoyed two weeks of near optimal growth conditions before the arrival of biological agents in this study. This lead to an overwhelming population boom that caused crop failure of the monoculture of 'Frillice' while moderately affecting the cultivars investigated in this study. The biological agents got the pests under control only after the harvests were discarded and a later population growth of biological agents.

It is also important to note that the research quality of these quantitative experiments would be improved if the statistics were based on a statistically significant sample size. This means that the results obtained from this study cannot be generalized, and should only be interpreted as indications or trends. The detailed description of materials and methods does however provide transparency strengthening the quality of this research.

6. Conclusions

The yield and fresh weight of leaves and roots of ‘Frillice’ produced in the preliminary monoculture study varied mainly in relation to environmental factors such as light intensity, temperature and changes in the available nutrient concentrations produced by fish and bacteria. The unusually cold and rainy weather conditions in September, combined with a decrease in nutrient production due to the harvesting of fish lead to the lowest harvest yields of ‘Frillice’ in Mid-September. An overall decreasing yield trend was observed as the study progressed into autumn.

ANOVA showed no significant difference between any growth parameter and the aquaponic and hydroponic systems ($p<0.05$), meaning that the aquaponic and hydroponic systems produced similar plants. High quality seedlings with long, thick roots penetrating the rockwool cubes was shown to be a good determining factor when predicting potential plant growth and development in a cold water aquaponic system. The germination procedure that was optimal for ‘Frillice’ proved unable to produce ‘Bulls Blood’ seedlings of satisfactory quality. It is important that germination procedures optimized for different plant species and cultivars are followed, researched or developed to ensure the best start of any future experiments.

The highest yields were obtained by the two lettuce cultivars ‘Salanova Excite R2’ and ‘Frillice’. A weak trend suggested that the hydroponic control resulted in higher fresh weights of ‘Salanova Excite R2’, ‘Frillice’ and ‘Bulls Blood’ than the aquaponic system. ‘Marino’ seemed to have a higher fresh weight when produced in the aquaponic system than the hydroponic control. The same trends were observed for yield per square meter. ANOVA showed no statistical significance of these trends ($p<0.05$). ‘Salanova Excite R2’ and ‘Frillice’ obtained the highest average leaf weights per cube while ‘Marino’ produced both the heaviest and longest plant roots ($p<0.05$). ‘Salanova Excite R2’ obtained the highest number of leaves, while ‘Marino’ obtained the tallest leaves ($p<0.05$).

The literature review showed that the plant yield of lettuce, coriander and swiss chard varied both with temperature and aquaponic system. The growth parameters of yield and fresh weight of leaves were the most comparable to results obtained by other studies. The yields of ‘Frillice’ from both the preliminary and comparative study were higher compared to lettuce yields reported by Sace & Fitzsimmons (2013) and Buzby (2016). The preliminary lettuce yield was higher than yields reported by Skar et al. (2015). The lettuce yield and fresh weights obtained in the comparative study were lower than all other lettuce yields shown in Table 2-6 of the literature study. ‘Marino’ yields were within the yield range reported in literature. ‘Bulls Blood’ yields in the comparative study were higher compared by Buzby et al. (2016). These results indicate that the cold water system at Landvik is able to produce plants that are comparable those produced in other cold water studies, while most warm water studies produced higher plant yields.

Brown trout is recommended for cold water aquaponic systems because it was superior to bleke in terms of specific growth rate and harvest weight. Rainbow trout (*Oncorhynchus mykiss*) and freshwater prawns (*Macrobrachium rosenbergii*) might also be good options for nutrient production in a cold water aquaponic system.

The nutrient concentrations in plants, fish and water were similar between the preliminary and comparative studies with mostly minor differences between cultivars and the aquaponic and hydroponic systems. Only Na concentrations were higher in both water and plants produced in the aquaponic system compared to the hydroponic control, with ‘Bulls Blood’ having the highest Na content. Satisfactorily low concentrations of ammonium, nitrite and nitrate allowed healthy fish production while satisfying the nutritional requirements of the examined plant cultivars. The aquaponic system was able to recover after a critical aphid infestation when biological agents got control. This resulted in a monoculture of ‘Frillice’ and a polyculture of four different plant cultivars free of nutrient deficiencies or severe pest damage.

These conclusions should serve as indications rather than almost certainties although the growth parameters seem comparable and similar to those reported by other cold water studies. This is because the statistics are based on a very limited sample size of 24 plants per replicate and cultivar in the aquaponic system, and 12 plants per replicate and cultivar in the hydroponic system.

Suggested research topics for further studies

- Investigations of different fish feed compositions that satisfy the nutritional requirements of both fish and plants without a nutrient deficiency or build-up in the aquaponic system water. Advances in this area would reduce or eliminate the need of nutrient supplementation in aquaponic production.
- Germination and root growth studies of the growth and establishment of additional coriander and swiss chard cultivars as well as other commercially viable plants. This would provide more data for comparisons of other plant species that are less studied than lettuce.
- Plant placement vs. growth. Is a placement closer to the influent producing higher plant yields than a placement closer to the effluent water due to higher nutrient concentrations in the influent water?
- Additional data on the tolerance of TAN, nitrite and nitrate of European lobster and other aquatic species would provide aquaponic producers with more choices when deciding on what species to use for a nutrient producer.

References

Al-Hafedh, Y. S., A. Aftab & Beltagi, M. S. (2008). Food Production and Water Conservation in a Recirculating Aquaponic System in Saudi Arabia at Different Ratios of Fish Feed to Plants. *Journal of the world aquaculture society* 39(4): 510-520.

Antoniou, P., Hamilton, J., Koopman, B., Jain, R., Holloway, B., Lyberatos, G. & Svoronos, S. A. (1990). Effect of temperature and ph on the effective maximum specific growth rate of nitrifying bacteria. *Water Research* 24(1): 97-101.

Arnon, D. I., & Stout, P. R. (1939). The essentiality of certain elements in minute quantity for plants with special reference to copper. *Plant Physiology* 14(2): 371-375.

Bathia, K. & Wasiim, M. (2012). *Ornamental fish as complementary species in aquaponics*. Master of Science in Aquaculture. Newfoundland, Memorial University of Newfoundland.

Bazzaz, F. A. & Sombroek, W. (Ed.) (1996). *The CO_2 fertilization effect: higher carbohydrate production and retention as biomass and seed yield* in *Global climate change and agricultural production. Direct and indirect effects of changing hydrological, pedological and plant physiological processes*. West Sussex, England, Food and Agriculture Organization of the United Nations (FAO) and John Wiley & Sons Ltd. Retrieved from http://www.fao.org/docrep/w5183e/w5183e06.htm, on 05.08.2016.

Becker, C. & Kläring, H-P. (2015). CO_2 enrichment can produce high red leaf lettuce yield while increasing most flavonoid glycoside and some caffeic acid derivative concentrations. *Food Chemistry* 199(May): 736-745.

Bioforsk. Radiation values retrieved from http://lmt.bioforsk.no/agrometbase/showweatherdata.php?weatherstation=29&elementMeasurementTypes%5B%5D=39&loginterval=3&valuetype=value_controlled&date_start=2015-08-01&date_end=2015-10-31&format=html&separator=comma&adjustForDST=true, 02.08.2016.

Blidariu, F. & Grozea, A. (2011). Increasing the Economical Efficiency and Sustainability of Indoor Fish Farming by Means of Aquaponics - Review. *Animal Science and Biotechnologies* 44(2): 1-8.

Blidariu, F., Radulov, I., Lalescu, D., Drasovean, A. & Grozea, A. (2013). Evaluation of Nitrate Level in Green Lettuce Conventional Grown under Natural Conditions and Aquaponic System. *Animal Science and Biotechnologies* 46(1): 244-250.

Buzby, K. M., Waterland, N. L., Semmens, K. J. & Lin, L-S. (2016). Evaluating aquaponic crops in a freshwater flow-through fish culture system. *Aquaculture* 460(2016): 15-24.

Bævre, O. A. & Gislerød, H. R. (1999). *Plantedyrking i regulert klima*. 2nd Ed. Valdres, Forfatterne og A/S Landbruksforlaget.

Dalsgaard, J., Lund, I., Thorarinsdottir, R., Drengstig, A., Arvonen, K. & Pedersen, P. B. (2012). Farming different species in RAS in Nordic countries: Current status and future perspectives. *Aquacultural Engineering* 53(2013): 2-13.

Dedryver, C.-A., Le Ralec, A. & Fabre, F. (2010). The conflicting relationships between aphids and men: A review of aphid damage and control strategies. *Comptes Rendus Biologies* 333(6-7): 539-553.

Det Kongelige Landbruks- og Matdepartement. (2011-2012). *Landbruks- og matpolitikken. Velkommen til bords.* (St.meld. nr. 9 2011-2012).

Encyclopædia Britannica. *Chinampa.* Retrieved from https://global.britannica.com/topic/chinampa on 20.04.2016.

Diver, S. (2006) Aquaponics - Integration of Hydroponics with Aquaculture. National Sustainable Agriculture Information Service (ATTRA). Retrieved from https://attra.ncat.org/attra-pub/summaries/summary.php?pub=56 on 03.02.2016.

Drost, D. (2010) Swiss Chard in the Garden. Retrieved from https://extension.usu.edu/-files/publications/publication/HG_Garden_2005-14.pdf on 09.08.2016.

Endut, A., Jusoh, A., Ali, N., Nik, W. B. W. & Hassan, A. (2009). A study on the optimal hydraulic loading rate and plant ratios in recirculation aquaponic system. *Bioresource Technology* 101(2010): 1511-1517.

Epstein, E., & Bloom, A. J. (2005). *Mineral Nutrition of Plants: Principles and Perspectives.* 2nd Ed. Sunderland, MA., Sinauer Associates.

Freidig, A. K. & Goldman, I. L. (2014). Geosmin (2β,6α-Dimethylbicyclo[4.4.0]decan-1β-ol) Production Associated with *Beta vulgaris* ssp. *vulgaris* Is Cultivar Specific. *Journal of Agricultural and Food Chemistry* 62(9): 2031-2036.

Graber, A. & Junge, R. (2008). Aquaponic Systems: Nutrient recycling from fish wastewater by vegetable production. *Desalination* 246(1-3): 147-156.

Hallam, M. (N.D.). “Murray Hallam’s Aquaponic Secrets” (video).

Hanson, A., Yabes Jr., J. & Primavera, L. P. (2008). Cultivation of Lemon Basil, Ocimum americanum, in two different hydroponic configurations supplemented with various concentrations of Tilapia aquaculture green water. *Bios* 79(3): 92-102.

Havlin, J. L., Beaton, J. D., Tisdale, S. L. & Nelson, W. L. (2005). *Soil Fertility and Fertilizers An Introduction to Nutrient Management.* Upper Saddle River, New Jersey, Pearson Education Inc.

Kaczperski, M. P., Carlson, W. H. & Karlsson, M. G. (1991). Growth and Development of *Petunia* x hybrids as a Function of Temperature and Irradiance. *Journal of the American Society for Horticultural Science* 116(2): 232-237.

Kimball, B. A. & Idso, S. B. (1983). Increasing Atmospheric CO_2: Effects on crop yield, water use and climate. *Agricultural Water Management* 7(1-3): 55-72.

Kotzen, B. & Appelbaum., S. (2010). An Investigation of Aquaponics Using Brackish Water Resources in the Negev Desert. *Journal of Applied Aquaculture* 22(4): 297-320.

Lenardis, A., de la Fuente, E., Hil, A. & Tubía, A. (1999). Response of Coriander (Coriandrum sativum L.) to Nitrogen Availability. *Journal of Herbs, Spices & Medicinal Plants* 7(4): 47-58.

Lennard, W. A. & Leonard., B. V. (2004). A comparison of reciprocating flow versus constant flow in an integrated, gravel bed, aquaponic test system. *Aquaculture International* 12(6): 539-553.

Lennard, W. A. & Leonard, B. V. (2006). A comparison of three different hydroponic sub-systems (gravel bed, floating and nutrient film technique) in an Aquaponic test system. *Aquaculture International* 14(6): 539-550.

LOG. *LOG Seed catalogue.* Retrieved from https://issuu.com/annecarlson0/docs/katalog_gr___nnsaker_2016_for_web?e=16848265/31722499 on 31.07.2016.

Majara, E. (2014). *Utilization of the Trends in the Horticultural Production - Well-being and Food Trends and Countertop Garden Devices (Trendien hyödyntäminen puutarhatuotannossa - Hyvinvointi- ja ruokatrendit sekä pöytäpuutarhat).* Bachelor in Horticulture, Lepaa, Finland, Häme University of Applied Sciences.

Mattson, N. S. & Peters, C. (N.D.) A Recipe for Hydroponic Success. Retrieved from http://www.greenhouse.cornell.edu/crops/factsheets/hydroponic-recipes.pdf, on 22.06.2016.

McCormick, S. D., Shrimpton, J. M., Carey, J. B., O'Dea, M. F., Sloan, K. E., Moriyama, S. & Björnsson, B. Th. (1998). Repeated acute stress reduces growth rate of Atlantic salmonn parr and alters plasma levels of growth hormone, insulin-like growth factor I and cortisol. *Aquaculture* 168(1-4): 221-235.

Morgan, L (2016). *Getting the Most from Hydroponic Basil and Cilantro.* Retrieved from http://maximumyield.com/blog/2016/05/30/hydroponic-basil-cilantro/, on 25.06.2016.

Myster, J. & Moe, R. (1995). Effect of diurnal temperature alternations on plant morphology in some greenhouse crops - a mini review. *Scientia Horticulturae* 62(4): 205-215.

Norges Forskningsråd (2006). *HAVBRUK - Produksjon av akvatiske organismer (2000-2005) Havbruksforskning: Fra merd til mat.* Oslo.

Palm, H. W., Seidemann, R., Wehofsky, S. & Knaus, U. (2014). Significant factors affecting the economic sustainability of closed aquaponic systems. Part I: system design, chemo-physical parameters and general aspects. *Aquaculture, Aquarium, Conservation & Legislation. International Journal of the Bioflux Society* 7(1): 20-32.

Pantanella, E., Cardarelli, M., Colla, G., Rea, E. & Marcucci, A. (2010). *Aquaponics vs. Hydroponics: Production and Quality of Lettuce Crop.* Proceedings of the 28th IHC- International Symposium on Greenhouse and Soilless Cultivation.

Petrea S. M., Cristea, V., Dediu, L., Contoman, M., Lupoae, P., Mocanu, M. C. & Coada, M. T. (2013a). Vegetable Production in an Integrated Aquaponic System with Rainbow Trout and Spinach. *Bulletin UASVM Animal Science and Biotechnologies* 70(1): 45-54.

Petrea S. M., Cristea, V., Dediu, L., Contoman, M., Lupoae, P., Mocanu, M. C., Coada, M. T., Ion, S. P., Mocanu, M. C. & Antache, A. (2013b). A Study of Nitrogen Cycle in an Integrated Aquaponic System with Different Plant Densities. *Bulletin UASVM Animal Science and Biotechnologies* 70(1): 55-64.

Rafiee, G. & Saad, C. R. (2004). Nutrient cycle and sludge production during different stages of red tilapia (*Oreochromis* sp.) growth in a recirculating aquaculture system. *Aquaculture* 244(2005): 109-118.

Rakocy, J. E., Bailey, D. S., Shultz, K. A. & Cole, W. M. (1997). *Evaluation of a Commercial-Scale Aquaponic Unit for the Production of Tilapia and Lettuce. Tilapia Aquaculture.* Proceedings of the Fourth International Symposium on Tilapia in Aquaculture, Orlando, Florida.

Rakocy, J. E., Shultz, R. C., Bailey, D. S. & Thoman, E. S. (2004a). Aquaponic Production of Tilapia and Basil: Comparing a Batch and Staggered Cropping System. Proceedings of SPSCC, International Society for Horticultural Science, *Acta Horticulturae* 648: 63-69.

Rakocy, J. E., Bailey, D. S., Shultz, R. C. & Thoman, E. S. (2004b). *Update on tilapia and vegetable production in the UVI aquaponic system.* Proceedings of the sixth International Symposium on Tilapia in Aquaculture, Orlando, Florida.

Rakocy, J. E., Masser, M. P. & Losordo, T. M. (2006) *Recirculating Aquaculture Tank Production Systems: Aquaponics - Integrating Fish and Plant Culture.* SRAC Publication No. 454. Retrieved from http://www.gemstone.umd.edu/team-sites/classof2014/mega/documents/Rakocy_RAS.PDF, on 18.11.2015.

Rakocy, J. E., Bailey, D. S., Shultz, R. C., & Danaher, J. J. (2011). *A Commercial-Scale Aquaponic System Developed at the University of the Virgin Islands*. Proceedings of the 9th International Symposium on Tilapia in Aquaculture.

Roosta, H. R. & Hamidpour, M. (2013). Mineral nutrient content of tomato plants in aquaponic and hydroponic systems: effect of foliar application of some macro- and micro-nutrients. *Journal of Plant Nutrition* 36(13): 2070-2083.

Sace, C. F. & Fitzsimmons, K. M. (2013). Vegetable production in a recirculating aquaponic system using Nile tilapia (*Oreochromis niloticus*) with and without freshwater prawn (*Macrobrachium rosenbergii*). *Academia Journal of Agricultural Research* 1(12): 236-250.

Savidov, N. A., Hutchings, E. & Rakocy, J. E. (2007). Fish and Plant Production in a Recirculating Aquaponic System: A New Approach to Sustainable Agriculture in Canada. International Conference and Exhibition on Soilless Culture, Leuven, Belgium. International Society for Horticultural Science (ISHS) *Acta Horticulturae* 742(28): 209-221.

Schoonhoven, L. M., Loon, J. J. A. & Dicke, M. (2005). *INSECT-PLANT BIOLOGY*. New York, U.S.A., Oxford University Press Inc.

Seawright, D. E., Stickney, R. R. & Walker, R. B. (1997). Nutrient dynamics in integrated aquaculture-hydroponics systems. *Aquaculture* 160(3-4): 215-237.

Sikawa, D. C. & Yakupitiyage, A. (2010). The hydroponic production of lettuce (*Lactuca sativa* L) by using hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*) pond water: Potentials and constraints. *Agricultural Water Management* 97(9): 1317-1325.

Silva, L., Gasca-Leyva, E., Escalante, E., Fitzsimmons, K. M. & Lozano, D. V. (2015). Evaluation of Biomass Yield and Water Treatment in Two Aquaponic Systems Using the Dynamic Root Floating Technique (DRF). *Sustainability* 7(11): 15384-15499.

Skar, S. L. G., Liltved, H., Kledal, P. R., Høgberget, R., Björnsdottir, R., Homme, J. M., Oddsson, S., Paulsen, H., Drengstig, A., Savidov, N. & Seljåsen, R (2015). *Aquaponics NOMA (Nordic Marine) New Innovations for Sustainable Aquaculture in the Nordic Countries.* Nordic

Innovation publication 2015:06. Retrieved from http://nordicinnovation.org/Global/_Publications/Reports/2015/P11090%20-%20Aquaponics%20RAPPORT%20-13%2001%2016.pdf on 21.08.2015.

Taiz, L. & Zeiger., E. (2010). *Plant Physiology*. MA, U.S.A., Sinauer Associates, Inc.

Terofal, F. / Torgersen, L. S. (1978/1980). *Fische. Unsere Süsswasser- und Meresfische nach Farbfotos bestimmen / Naturguide Fisker De vanligste ferskvanns- og saltvannsfisker*. München / Norway, BLV Verlagsgesellschaft mbH / NKS-Forlaget.

Tyson, R. V. (2007). *Reconciling pH for ammonia biofiltration in a cucumber/tilapia aquaponics system using a perlite medium.* Doctor of Philosophy. Florida, University of Florida.

Tyson, R. V., Treadwell, D. D. & Simonne, E. H. (2011). Opportunities and Challenges to Sustainability in Aquaponic Systems. *HortTechnology* 21(1): 6-13.

Verma, A., Pandeya, S. N., Yadav, S. K., Singh, S. & Soni, P. (2011). A review on Coriandrum sativum (Linn.): an ayurvedic medicinal herb of happiness. *Journal of Advances in Pharmacy and Healthcare Research* 1(3): 28-48.

Vermeulen. T. & Kamstra, A. (2013). The Need for Systems Design for Robust Aquaponic Systems in the Urban Environment. International Symposium on Soilless Cultivation, ISHS *Acta Horticulturae* 1004(6): 6-13.

Whiting, D., O'Meara, C. & Wilson, C. (2014) *Vegetable Planting Guide*. Colorado State University. Retrieved from http://www.ext.colostate.edu/mg/gardennotes/720.html#s-emihardy, on 09.08.2016.

Yara a: Fertilizer recommendations: http://www.yara.no/gjodsel/vekster/andre-vekster/Salat/Salat.aspx and http://www.yara.no/gjodsel/vekster/andre-vekster/Rodbete/ on 15.07.2016.

Yara b: Nutrient solution values calculated from *http://www.yara.no/gjodsel/sortiment/other/3531-kristalon-indigo/* and *http://www.yara.no/gjodsel/sortiment/yaraliva/3540-yaraliva-calcinit/* on 06.06.2016.

Yr: Temperature and precipitation data retrieved from http://www.yr.no/sted/Norge/Aust-Agder/Grimstad/Landvik~7679/statistikk.html, 02.08.2016.

Zhu, S. & Chen., S. (2002). The impact of temperature on nitrification rate in fixed film biofilters. *Aquacultural Engineering* 26(4): 221-237.

Appendixes

Appendix A: Experimental set-up of hydroponic and aquaponic plant production in the comparative study

The following table shows the experimental set-up of the comparative study. Four aquaponic replicates were placed in the southern most part of the western plant tank shown in Figure 3-1. The replicates shared the plant tank with the continuing monoculture production of 'Frillice' shown in gray.

Replicate 1	Replicate 2	Frillice in monoculture				
Replicate 3	Replicate 4					

The following tables show the experimental set-up of the randomized placement of cultivars into the hydroponic control and the aquaponic system. The hydroponic replicates contained three plants of each of the four cultivars while the aquaponic replicates contained six plants of each cultivar. The left side of the tables correspond with a southern orientation and the right side of the tables correspond with a northern orientation, (S → N).

The hydroponic control

Replicate 1		Replicate 2		Replicate 3	
Bulls Blood	Frillice	Frillice	S. Excite R2	S. Excite R2	Bulls Blood
S. Excite R2	S. Excite R2	Marino	S. Excite R2	Frillice	Marino
Bulls Blood	Bulls Blood	Marino	Frillice	Frillice	Marino
S. Excite R2	Marino	Bulls Blood	Frillice	S. Excite R2	Frillice
Frillice	Marino	Marino	Bulls Blood	Bulls Blood	S. Excite R2
Frillice	Marino	Bulls Blood	S. Excite R2	Marino	Bulls Blood

The Aquaponic system

Replicate 1:

Marino	Marino	Frillice	Frillice
Salanova Excite R2	Marino	Salanova Excite R2	Bulls Blood
Frillice	Marino	Marino	Frillice
Bulls Blood	Bulls Blood	Marino	Salanova Excite R2
Salanova Excite R2	Salanova Excite R2	Bulls Blood	Frillice
Bulls Blood	Salanova Excite R2	Frillice	Bulls Blood

Replicate 2:

Salanova Excite R2	Bulls Blood	Bulls Blood	Salanova Excite R2
Bulls Blood	Frillice	Salanova Excite R2	Marino
Frillice	Frillice	Frillice	Marino
Salanova Excite R2	Marino	Marino	Bulls Blood
Frillice	Frillice	Salanova Excite R2	Marino
Bulls Blood	Salanova Excite R2	Bulls Blood	Marino

Replicate 3:

Marino	Frillice	Marino	Salanova Excite R2
Frillice	Marino	Marino	Frillice
Marino	Salanova Excite R2	Frillice	Frillice
Bulls Blood	Bulls Blood	Salanova Excite R2	Salanova Excite R2
Bulls Blood	Salanova Excite R2	Frillice	Bulls Blood
Salanova Excite R2	Marino	Bulls Blood	Bulls Blood

Replicate 4:

Salanova Excite R2	Marino	Marino	Bulls Blood
Salanova Excite R2	Frillice	Salanova Excite R2	Frillice
Bulls Blood	Marino	Bulls Blood	Salanova Excite R2
Marino	Frillice	Frillice	Bulls Blood
Frillice	Frillice	Bulls Blood	Salanova Excite R2
Marino	Marino	Salanova Excite R2	Bulls Blood

Appendix B: An overview of the fish and plants used in aquaponic studies

*Table 0-1: An overview that shows which fish species that have grown together with which plant species and in what temperature and pH-range. The numbers in parenthesis indicate what reference the data was found in. N.D. = No data. * = The lower temperature was recorded in a system with arctic char while the higher was recorded in a system with tilapia. The study by Skar et al. (2015) consisted of three different studies.*

Fish species	**Plant species or cultivar**	**Water temperature**	**pH-value**	**References**
African catfish (*Clarias gariepinus)*	Water spinach (*Ipomoea aquatica*)	27.5-28.8	5.6-7.3	Endut et al., 2010
Arctic char (*Salvelinus alpinus*)	Basil (*Ocimum basilicum*) Dill (*Anethum graveolens*) Lettuce (*Lactuca sativa*) Mizuna (*Brassica rapa nipponosica*) Parsley (*Petroselinum crispum*) Rocket (*Eruca sativa*) Spinach (*Spinacia oleracea*) Tomato (*Solanum lycopersicum*)	16.3-32*	6.9-7.0	Skar et al., 2015
Brown trout (*Salmo trutta*)	Coriander (*Coriandrum sativum*) Lettuce (*Lactuca sativa*) Strawberry (*Fragaria × ananassa*) Swiss chard 'Bulls Blood'	16.3-32	6.9-7.0	Skar et al., 2015
Common carp (*Cyprinus carpio*), Grass carp (*Ctenopharyngodon idella*), Silver carp (*Hypophthalmichthys molitrix*)	Tomato (*Solanum lycopersicum*) cultivar 'Blizzard'	22.7	7.0-7.7	Roosta & Hamidpour, 2013
Eurasian perch (*Perca fluviatilis*)	Cucumber (*Cucumis sativus*)	N.D.	6.8-7.8	Graber & Junge, 2009

	Tomato (*Solanum lycopersicum*)			
Freshwater prawn (*Macrobrachium rosenbergii*)	Chinese cabbage (*Brassica rapa pekinensis*) Lettuce (*Lactuca sativa*) Pac choi (*Brassica rapa*)	16.3-24.0	6.5-8.2	Sace & Fitzsimmons, 2013
Hybrid catfish (*Clarias microcephalus* x *C. gariepinus*)	Lettuce (*Lactuca sativa* L.)	28.9-30.9	7.1-7.4	Sikawa & Yakupitiyage, 2010
Murray Cod (*Maccullochella peelii peelii* (Mitchell))	Green oak lettuce (*Lactuca sativa*)	22.00	6.7-7.0	Lennard & Leonard, 2004; Lennard & Leonard, 2006
Pike perch (*Sander lucioperca*)	Lettuce (*Lactuca sativa*)	23.1-26.2	6.6-6.8	Blidariu et al., 2013
Platy (*Xiphophorus maculatus*), Goldfish (*Carassius auratus*)	Basil (*Ocimum basilicum*) Spinach (*Spinacia oleracea)* Watercress (*Nasturtium officinale)*	18.5-28.5	6.5-8.6	Bathia & Wasiim, 2012
Rainbow Trout (*Oncorhynchus mykiss*) (1,2,3,4)	Asian greens (Mizuna, Tokyo Bekana, Vitamin Green, Shungiku, Tatsoi) (3) Basil (*Ocimum basilicum*) (4) Chives (3) Cilantro (3) Dill (*Anethum graveolens*) (4) Green Shiso (3) Italian Oregano (3) Kolrabi (3)	16.8 (1) 16.2-17.8 (2) 13 (3) 16.3-32 (4)*	6.9 (1) 6.6-8.0 (2) 6.9-7.0 (4)	Petrea et al., 2013a (1); Petrea et al., 2013b (2); Buzby et al., 2016 (3); Skar et al., 2015 (4)

	Lettuce (*Lactuca sativa*) (3) Lovage (3) Mizuna (*Brassica rapa nipponosica*) (4) Mustard greens (3) Other greens (Minutina, Cress, Arugula, Italian dandelion) (3) Parsley (*Petroselinum crispum*) (3, 4) Rocket (*Eruca sativa*) (4) Rosemary (3) Salad Burnet (3) Sorrel (3) Spinach (*Spinacia oleracea*) (1,2) Swiss chard (3) Swiss chard ‘Bulls Blood’ (3) Tomato (*Solanum lycopersicum*) (4) Winter Savory (3)			
Tilapia (*Oreochromis niloticus*) (1,2,3,4,5,6,7,8,9,10,11,12), Red tilapia (3), red ear sunfish (*Lepomis microlophus*) (3)	Amaranth (*Amaranthus* sp.) (6) Aubergine (*Solanum melongena*) (1,5,6) Basil (*Ocimum basilicum*) (3,5,12) Bitter melon (*Momordica charantia*) (6) Broccoli (*Brassica oleracea* var. *italica*) (5)	N. D. (1) 22.2 (2) 24.0-29.0 (3) 24.0-29.0 (4) 22.6-27.9 (5) 24.8 (6) 16.3-24.0 (7) 27.0-32.0 (8) 25.1-27.5 (9) 25.5-29.6 (10) N.D. (11) 16.3-32 (12)	6.8-7.8 (1) 6.8-8.0 (2) 6.8-7.4 (3) 6.9-7.6 (4) 7.5-8.2 (5) 6.5-7.0 (6)	Graber & Junge, 2009 (1); Tyson, 2007 (2); Rakocy et al., 2004a (3); Rakocy et al., 2004b (4); Palm et al., 2014 (5); Savidov et al., 2007 (6); Sace & Fitzsimmons, 2013 (7);

	Cantaloupe (*Cucumis melo* var. *cantalupensis*) (9) Chinese cabbage (*Brassica rapa pekinensis*) (7) Chives (*Allium schoenoprasum*) (5,6,9) Cilantro (*Eryngium foetidum*) (6) Coriander (*Coriandrum sativum*) (6) Cucumber (*Cucumis sativus*) (2,5,6,9) Dill (*Anethum graveolens)* (6) Fenugreek (*Trigonella foenum-graecum*) (6) Lettuce (*Lactuca sativa*) (5,7,8,9,10,11,12) Mizuna (*Brassica rapa nipponosica*) (12) Okra (*Abelmoschus* esculentus) (3,9) Pac choi (*Brassica rapa*) (7) Paprika (*Capsicum annum*) (5) Parsley (*Petroselinum crispum*) (6) Peppermint (hybrid *Mentha* x *piperita*) (5) Purslane (*Portulaca oleracea*) (6)		6.5-8.2 (7) 7.5 (8) 7.2 (9) 7.7-8.3 (10) 6.5-7.0 (11)	Seawright et al., 1997 (8); Rakocy et al., 2011 (9); Al-Hafedh et al., 2008 (10); Pantanella et al., 2010 (11); Skar et al., 2015 (12)

	Rocket (*Eruca sativa*) (12) Rosemary (*Rosmarinus officinalis*) (5) Spinach (*Spinacia oleracea*) (5,6,12) Swiss chard (*Beta vulgaris subsp. vulgaris*) (6) Tomato (*Solanum lycopersicum*) (5,6) Water cress (*Nasturtium officinale*) (6) Water spinach (*Ipomoea aquatic*) (6) Zucchini (*Cucurbita pepo*) (5)			

Appendix C: Figures containing climatic data from the weather station at Landvik

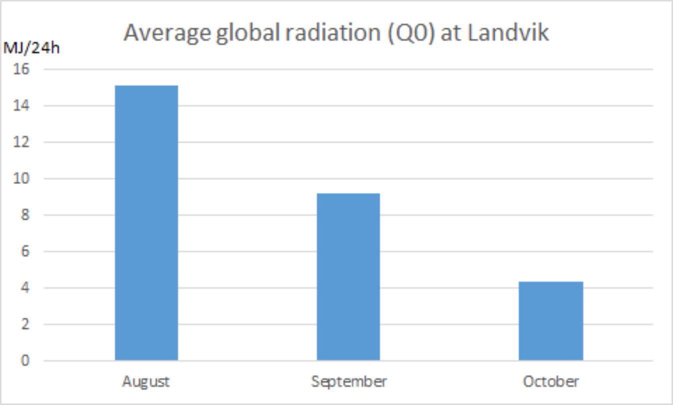


Figure 0-1: Average global radiation per 24h during the preliminary monoculture study of 'Frillice' (Bioforsk).

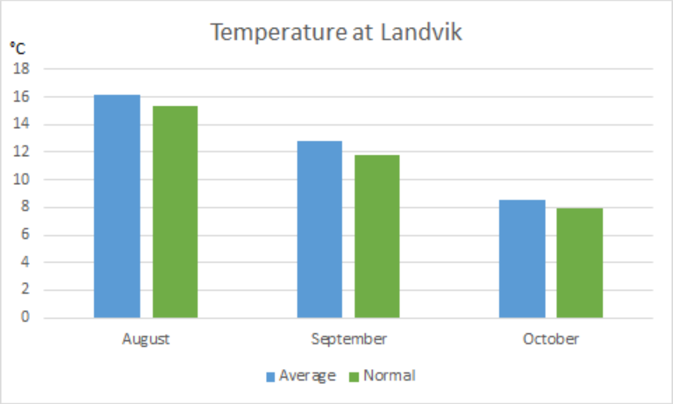


Figure 0-2: Current average and normal temperatures measured by the NIBIO weather station at Landvik during the preliminary monoculture study of 'Frillice' (Yr).

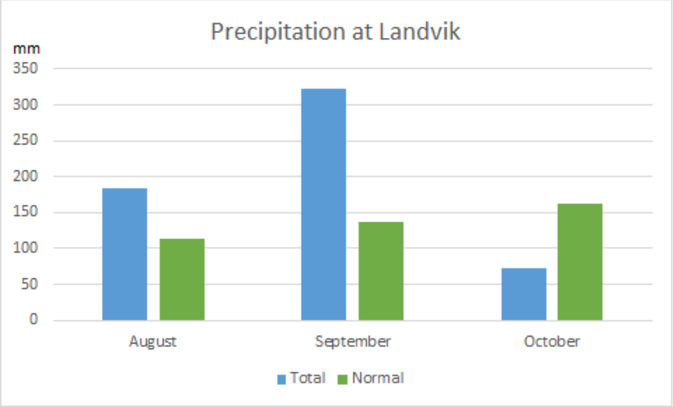


Figure 0-3: Total 2015 and normal precipitation measured by the NIBIO weather station at Landvik (Yr).

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