Abstract

Atlantic salmon (*Salmo salar)* is the main fish species harvested from aquaculture, making up a total of 93,9% of Norway's total fish production. Expansion of aquaculture has over the last few years proved difficult due to environmental and geographical challenges, and alternative production methods are being investigated. Recirculating aquaculture systems (RAS) has proved to be a viable alternative but has faced problems with acute mortality events suspected to be caused by hydrogen sulfide. Hydrogen sulfide is produced by oxidation of sulfate, and a proposed possible solution this problem is to desalinate seawater with a nanofiltering membrane, specifically designed to remove sulfate.

The aim of this thesis was to study the effects a nanofiltering membrane has on the composition of seawater and see if sulfur content can be removed without changing other key parameters. Over the course of 13 weeks samples where taken at 10 different points on two RAS. One with nanofiltered inlet water and one with a combination of seawater and freshwater to serve as control. These samples where then measured for pH, salinity and conductivity, and analyzed by ICP-MS, IC and UV-vis.

The main objective of this thesis was to find the effiency of sulfate removal by nanofiltering membrane, investigated by analysis with ICP-MS and IC. Sulfur content had a reduction of 93,37 % measured by ICP-MS and had a reduction of 85,85% when compared to a control. The second objective was to see the effect on water quality parameters as well as other key ions. pH saw an increase by 1,41 % before and after nanofilter, but there was no significant difference between treated water an control. There was a 60,13 % reduction of salinity and 56,83 % reduction of conductivity after nanofiltration, but no significant difference when compared to control. Magnesium, silicone, potassium, calsium, bromide and strontium all saw a reduction after treatment, with magnesium, silicone, calsium and strontium having a lower concentration when compared to control, while potassium and bromide had a higher concentration.

Overall, this work demonstrated that use of nanofiltering membrane can achieve a large reduction of sulfur content, while keeping salinity, pH and conductivity unchanged in comparison to control. However, there were some observed changes in other ions, and further investigations are needed to determine the effects this has on health and wellbeing of Atlantic Salmon.

Sammendrag

Atlanterhavslaks (*Salmo salar*) er den største fiskearten som produseres i akvakulturnæringen, og representerer 93,39% av Norges totale fiskeproduksjon. Utvidelse av akvakulturdrift har i løpet av de siste årene vært problematisk, grunnet miljøhensyn og geografiske utfordringer. Resirkulerende akvakultur anlegg (RAS) has vist seg å være et godt alternativ, men har over de siste årene møtt på problemer med akutte massedødstilfeller hvor det mistenkes at hydrogen sulfid had spilt en stor rolle. Hydrogen sulfid oppstår etter oksidasjon av sulfat, og en spekulert løsning til dette problemet er å avsalte saltvann med et nanofilter som er spesifikt designet til å redusere sulfatinnhold.

Bakgrunnen denne oppgaven var å studere effekten nanofiltering har på komposisjonen av saltvann, og undersøke hvorvidt svovelinnhold kan reduseres uten at andre viktige vannkvalitetsparametere blir endret. I løpet av 13 uker ble det hentet ut prøver fra 2 ulike RAS anlegg. En av disse med nanofiltrert inntaksvann, og en med kombinasjon av saltvann og ferskvann som kontroll. Disse prøvene ble målt for pH, salinitet og konduktivitet, og analysert med ICP-MS, IC og UV-Vis.

Hovedformålet var å finne hvor effektiv nanofiltering kan fjerne svovelinnhold, som ble undersøkt med ICP-MS og IC. Det ble funnet en 93,37% reduksjon av svovel etter nanofiltering, og en 85,85% reduksjon sammenlignet med kontroll. Det andre formålet med oppgaven var å undersøke effekten på viktige vannkvalitetsparametere, samt undersøke effect på viktige ioner. pH økte med 1,41% etter nanofiltering, men det ble ikke funnet en signifikant forskjell sammenlignet med kontroll. Salinitet og konduktivitet ble redusert med henholdsvis 60,13 % og 56,83%, men det ble heller ikke her funnet en signifikant forskjell sammenlignet med kontroll. Magnesium, silitsium, kalium, kalsium, brom og strontium hadde alle en reduksjon etter nanofiltering, hvor magnesium, silitsium kalsium og strontium hadde en observer lavere konsentrasjon sammenlignet med kontroll, og kalsium og brom hadde en observer høyere konsentrasjon.

Dette arbeidet demonstrerte at bruk av nanofilter kan oppnå en høy reduksjon av svovelinnhold uten å påvirke vannkvalitetsparametere som pH, salinitet og konduktivitet. Det var forøvrig observer endring i andre ioner, og det behøves mer forskning for å finne ut av hvordan dette kan påvirke helsen og velværet til Atlanterhavslaks.

Acknowledgements

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Acronyms, abbreviations and symbols

RAS	Recirculating aquaculture system
NF	Nanofiltration
SRB	Sulfate reducing bacteria
TFC	Thin film composite membrane
LBL	Layer-by-layer
PE	Polyelectrolyte
SW	Spiral Wound
HF	Hollow-fibre
ICP-MS	Inductively coupled plasma mass spectrometry
IC	Ion Chromatrography
UV-vis	Ultraviolet-visible spectroscopy
I	Intensity
T	Transmittance
A	Absorbance
C	Concentration
ε	Molar absorption coefficient
d	Path length
σ	Standard deviation

1 Introduction

In 2019 the farming of Atlantic salmon (*Salmo Salar)* represented 93,9% of Norway's total aquaculture where a total of 1 364 044 tonnes were produced (Statistics Norway 2020), making Norway the biggest producer worldwide (Iversen et.al 2020). Aquaculture is an important part of Norwegian culture and economy, and it is estimated that there is room to increase total revenue by up to 6 times (Ministry of Trade, Industry and Fisheries 2014-2015). To achieve this there needs to be innovation in method of production, since ocean fisheries are facing both environmental and area challenges (Olaussen,J. 2018). One possible solution is a recirculating aquaculture system (RAS), where the outlet water from the fish tanks is recycled and reintroduced into the system, instead of being released into recipient water body done in conventional flow-through-systems (FTS). (Lekang, O. 2020 p. 257). RAS provides several benefits. Re-use of water will limit the demand for new water, making it possible to establish production in otherwise geologically challenging areas. Reduction of new water needed will also reduce energy costs and cleaning costs, since the overall body of water that needs to be treated is less. Lastly, a RAS can work around a poor water supply. Access to fresh water can be difficult and costly, and in RAS this can be bypassed by either combining freshwater and seawater, or perhaps desalinating seawater.

Still, there are some challenges. Operating RAS is still more energy-intensive than ocean fisheries and establishing a production site will require a high investment cost. There is also a problem with accumulation of waste products. Organic matter and particles from uneaten feed, feces and bacteria will cause gill irritation and stress (Chiam,C.K. et. Al. 2011) and build-up of ammonia, CO_2 and hydrogen sulfide (H_2S) can all cause harm and potential mortality events (Lekang, O. 2020 p. 264-26). Therefore, each RAS needs to install proper water treatment components further increasing the overall establishment cost. Hydrogen sulfide is the main suspect of many acute mortality events where feedwater has been a combination of saltwater and freshwater. Saltwater has a naturally high sulfate content (2700 mg/L, freshwater around 2 mg/L)(Boyd,C. 2014), and H_2S is a product of sulfate reduction (Holmer, M. and Storkholm,P. 2001) . A solution to this problem is still up for debate.

1.1 Aims and objectives

This master thesis is a part of a collaboration project between Nofima, Akvafresh and NTNU called Akvafresh, where the aim is to investigate the effects of using a nanofiltering membrane to desalinate saltwater used as feed water in RAS. More specifically, the objectives were to see if:

- Sulfate content can be reduced by nanofiltration to levels below 15 mg/L.

- Salinity will be kept at approximately 12 ‰

- Document effects on health and fitness, as well as documenting new water composition

For this thesis the aim was to document the effects of nanofilteration on water composition, specifically on pH, salinity, conductivity, and the changes in key ions. Two RAS provided by Nofima at the Nofima Centre for Recirculation in aquaculture where compared. One with desalinated water by nanofilter provided by Akvafresh, and one with combination water that served as a control. A full project description is included in appendix A.5.

2 Recirculating aquaculture systems

In RAS, the main principle is to recycle outlet water from the fish tank continuously instead of discarding it in a more conventional FTS, demonstrated in figure 2.1. Since FTS requires a steady water supply a lot of energy and cost is spent in temperature control, and choice of location becomes limited. RAS provides a different approach to aquaculture. With a constant recycling process total water consumption is reduced, decreasing energy costs at the same time. RAS is also preferable in implementation, as it can be designed to work with freshwater, brackish water and seawater (Lekang,O. 2020 p.267). Since it is a recirculating system, it does require a thorough water treatment, as there are many components that when accumulated can cause harmful effects.

Large particles, usually accumulating from untouched fish feed and feces, can cause harm to gills (Pedersen et al 2011) as well as being substrates for unwanted bacteria (Attramadal et al 2012). These are removed by a mechanical filter. Due to respiration, there will be a build-up of CO_2 throughout the life cycle of the fish. Long term exposure to high levels of CO_2 will have a negative effect on growth and fitness (Aslam et al 2019), as well as impacting pH. Therefore, control of CO_2 is usually done by a degasser where CO_2 rich water flows from the top of a stripping tower, while air flows the opposite direction from the bottom. This crosscurrent maximises fluid surface allowing CO_2 to escape and can reintroduce oxygen to the system if needed.
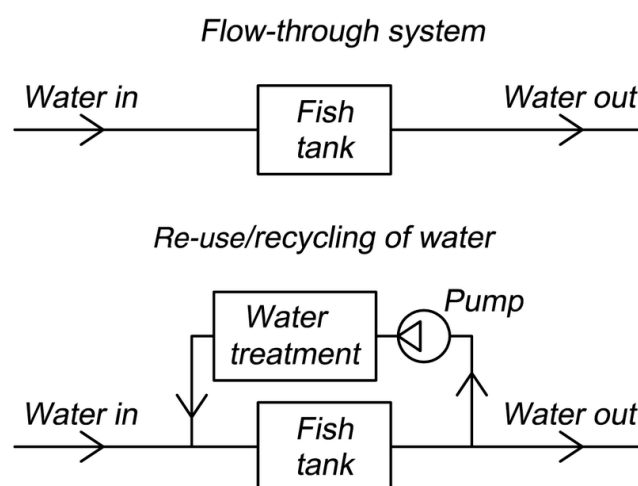
Figure 2.1 Flow through system compared with a recirculating aquaculture system. (Lekang,O. Aquaculture engineering (p. 285))

Last point of treatment is the removal of total-ammonia-nitrogen (TAN). TAN is the product of the protein metabolism and is secreted from the gills of the fish. When dissolved in water, TAN is present as both ammonia and ammonium. Ammonia is toxic and will with increased levels cause harmful effect (Iwama et al 1998). Therefore, TAN is commonly removed by utilizing a biofilter. A biofilter is a bed of media where bacteria grow to form a biological layer called a biofilm (Molleda et al 2008). The layer is populated by two groups of nitrifying bacteria, Nitrosomas and Nitrobacter, which will concrete ammonium to nitrite to nitrate. A simplification of this is given in equation 2.1. Efficiency of biofiltration depends primarily on temperature and pH, where optimal conversion happens between a pH of 7 and 9 (Noble et al 2018).

$$NH_3 \xrightarrow{Nitrosomas} NO_2^- \xrightarrow{Nitrobacter} NO_3 \qquad\qquad (2.1)$$
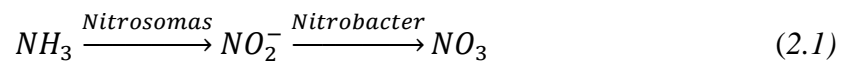
2.1 Water quality parameters

To ensure optimal growth conditions a RAS must adhere to many water quality parameters. This thesis will focus mainly on pH, salinity, and ion composition based on recommended values for Atlantic salmon.

2.1.1 pH

pH is an important parameter to monitor, as it will not only affect the efficiency of nitrification in biofilter, but will also have an effect on aluminium dissolved in water (Lydersen, E. 1990) Under alkaline conditions aluminium will have to toxic effect on Atlantic salmon (Poleo,A., and Hytterød,S. 2003), but under acidic conditions inorganic monomeric Al species will occur which is considered to be the most toxic to Atlantic salmon. The recommended pH levels according to the Norwegian Food Safety Authority is between 6,2 and 7,8, however if optimal nitrification is also a priority pH should be between 7,0 and 7,8.

2.1.2 Salinity

Salinity is defined as the mass in grams of salts in 1 kg of seawater, with major ion compositions given in table 2.1 (Byrne et al 2014). It affects other parameters like ammonia, dissolved oxygen, efficiency of nitrification in biofilter as well as efficiency of removal of CO_2 (Noble et al 2018), and it is recommended to have levels around 12 ‰ for cost-efficient production (Ytrestøyl et al. 2014)

Table 2.1 Summary of major ion composition found in seawater altered to mg/L (Byrne et al 2021)

Major ion composition, seawater (mg/L)	
Chlorine	18 980
Sodium	10 556
Sulphate	2 649
Magnesium	1 262
Calcium	400
Potassium	380
Bicarbonate	140
Bromide	65
Borate	26
Strontium	13
Fluoride	1
Silicate	1
Iodide	<1
Others	-
Total dissolved solids	**34 483**

To achieve optimal growth conditions in RAS it is important that the water provides the right ion composition, as many ions play an important part in fish metabolism and can only be accessed through gill intake. Magnesium acts as an activator of cofactors in enzyme systems (El-Mowafi, A. 1998), calcium is important for cellular signalling (Clapham,D. 2007) and potassium is important in maintenance of cellular volume and generation of nerve impulses (Kalantarian et al 2013). Keeping track of these ions is therefore an important part of a RAS process, to provide optimal growth condition and fish welfare.

2.1.3 Hydrogen sulfide, its accumulation and harmful effects on Atlantic salmon

Hydrogen Sulfide (H_2S) has been a much-debated topic in the salmon farming industry as of late, being the main suspect of several mass mortality events that has happened in RAS. Is is highly toxic to Atlantic salmon, and when digested it will cause respiratory failure (Forgan, L. and Donald, J. 2016). Sulfur is an essential element for life and is found in both seawater and freshwater in the form of either dissolved sulfate or sedimentary minerals. Sulfate mostly comes from the degradation of sulfur-containing rocks, or from the oxidation of organic sulphur found in decomposition of living things and waste products (Holmer, M. and Storkholm, P. 2001).

Concentration of sulfate in freshwater and seawater differ widely however, where concentrations are around 2 mg/L and 2700 mg/L respectively (Boyd,C. 2014).

H_2S is mostly made through sulfate reducing bacteria (SRB), found in anaerobic slime layers called biofilms. Biofilms are common in nature and grow at the water/solid interface in most biological systems (King et al 2008). It consists of essentially two layers: An aerobic top layer and an anaerobic bottom layer. The top layer consists of nitrifying bacteria (Schramm et al 2000) that converts ammonia and ammonium released from the fish into nitrate. The bottom layer is where SRB are found, and it is here that H_2S is created. There are multiple bacterial species involved in reduction of sulphate, mainly Sporovibrio desulfuricans and Desulfovibrio delsulfuricans. These bacteria are strictly anaerobic and use organic substances as electron donors and sulphate as electron acceptor, making conversion possible only when both sulphate and organic substances are available. The biofilm pathway starts with the nitrifying bacteria, that will convert all available anomia and ammonium in the water to nitrate, where nitrate is stored in the top layer structure keeping the anaerobic biofilm separated from the water. In the bottom layer two things are happening. Firstly, organic material and sulphate is converted into H_2S. Secondly, H_2S together with nitrate found in the upper layer is converted back to sulfate. This process will keep circulating until there is no more nitrate containing compounds left in the water. Once this happens the bottom layer of the biofilm will keep converting nitrate, but this time the structural integrity of the upper layer will be weakened until it disappears. This will release the underlying H_2S found in the bottom layer, which in large enough quantities could be harmful and even lethal to the fish.

Combatting the H_2S problem has been at the forefront of the RAS industry for many years, and different solutions have been proposed. According to Langeteig (2019) one possible solution to keeping levels of H_2S low is having sufficient levels of nitrate in the water to keep the biofilm at steady state. Since nitrate levels depends on the amount of ammonia and ammonium, which stems from feed and fish waste, the natural occurrence will fluctuate. Therefore, it is important to monitor nitrate concentrations and add a nitrate source when needed. This will prevent the bottom layer of the biofilm from degrading the upper layer, keeping the H_2S safely trapped.

Another suggested solution is to keep vigorous cleaning routines. Since biofilms occur on all surfaces, not only in the biofilter, but the idea also that removing the biofilm with sanitizers could be a possible solution. However, according to Schramm et al (2000), this could potentially eliminate beneficial nitrifying bacteria, which are essential to remove harmful build-up of ammonia. Some aspects of the system will need regular cleaning, depending on the system

design. Some areas of a RAS are more susceptible to "dead-zones", where a biofilm build up can get out of control. These zones are usually a result of bad circulation due to sharp corners or non-uniform water flow (Portz et al 2006) and will need further monitoring. An argument can be made that designing a RAS with a uniform flow in mind.

The removal of sulfate from inlet water is another approach. H_2S is seen mostly accumulating in RAS where inlet water has been partly or mainly seawater. Using freshwater would be beneficial since it is naturally low on sulfate, but it could prove to be difficult since access to freshwater is geographically restricted. However, a new solution of removing sulfate from seawater could be favourable. This would assure both low concentration of H_2S and easy access to inletwater, as saltwater is much more readily available than freshwater. This can be achieved by nanofiltration, a membrane separation process.

2 Nanofiltration

A membrane separation is defined as a thin sheet, film, or layer, which selectively separates components based on size or chemical attributes between two phases of either liquid, gas or vapor (Saleh, T. and Gupta, K. 2016). A typical membrane will have inlet for the compound that is getting separated (feed), an outlet for separated substance (permeate) and an outlet for the retained substance (retentate) showed in figure 2.1 (Droas, M. 2019).
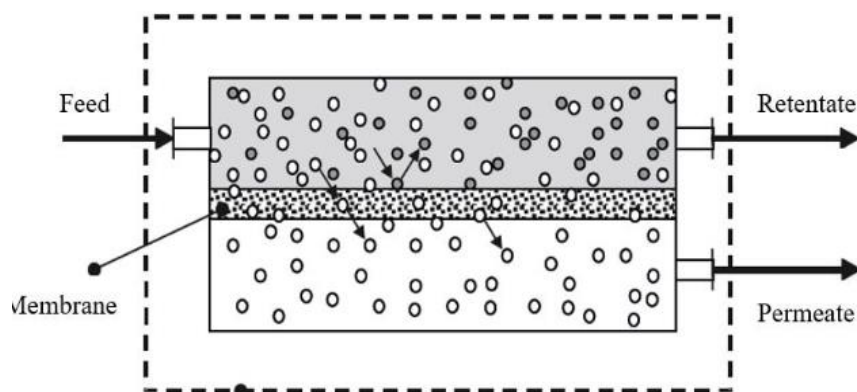
Figure 3.1 Graphical demonstration of the process behind general membrane separation (Graphics from: Droas,M. 2019)

Nanofiltration (NF) is the fourth class of pressure-driven membranes born after microfiltration (MF), ultrafiltration (UF) and reverse osmosis (RO) (Jye, L. and Ismail, A 2019). The different classes are defined by what kind of compounds they can separate based on size, chemical attribute, or operating pressure gradient, demonstrated in figure 3.2. There are three different

transport mechanisms at work in a NF membrane. Diffusion convection and electrostatic interactions. Diffusion is the transport of a compound between areas of high and low concentrations through a concentration gradient, convection is transport of a group of molecules through flow, and electrostatic interactions are movement caused by electrical charges. When selecting and designing a membrane separation process, all three of these transport mechanisms must be considered when choosing category and materials of the membrane.
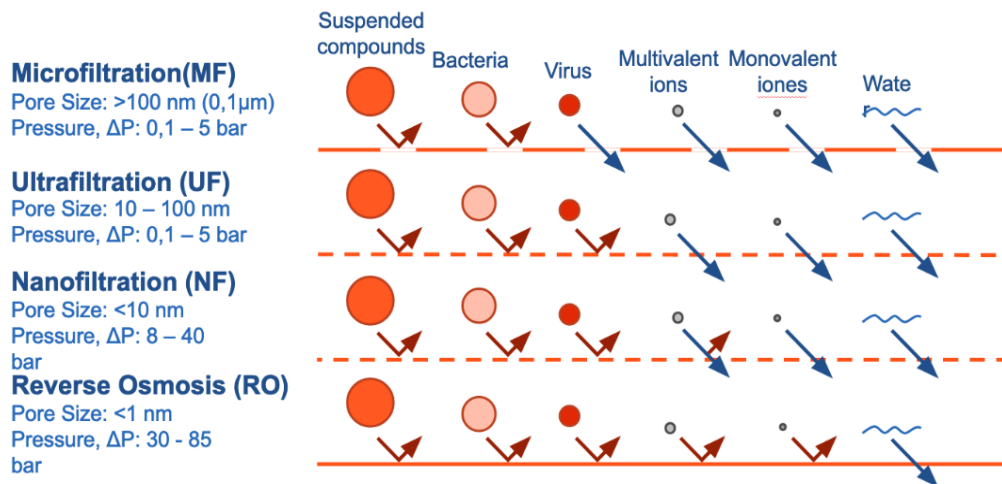
Figure 3.2 The four different classes of membrane separations, with pore sizes, operation pressures and an overview of types of compound that can be retained or separated. (Graphics provided by Akvafresh, 2020)

There are currently three main methods to synthesize NF membranes: (1) Poly amide thin film composite membrane (TFC), (2) single-step phase inversion and (3) layer-by-layer (LBL). An TFC synthesized membrane is an ultrathin selective poly amide (PA) layer, formed over the surface of a microporous support membrane. This creates what is known as a thin-film composite membrane (TFC). In brief, a microporous membrane made of either polysufone or polyethersulfone is treated with an amine monomer. This will start the formation of the PA layer, where the amine monomer is now fused to the top of the support membrane. The support is only there for mechanical strength, the separation is determined by the PA layer (Pinnau, I. 2000). The performance of the THC membrane, performance will vary with particle sizes, hydrophilicity, charge properties and pore channels of the materials used.
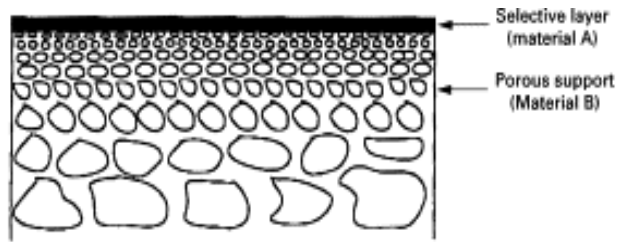
Figure 3.3 Representation of the selective PA layer and the microporous support layer of a thin film composite membrane. (Graphics from: Pinnau, I. 2000

The phase inversion method transforms a polymer solution from a liquid state to a solid state. This can be achieved several different ways. Immersion of polymer in bath of non-solvent, hot polymer solution casted over a chilled film, exposing the polymer to a vapour of a non-solvent, or evaporating a solvent from a polymer solution. The cheapest and therefore the most common option is the immersion method, demonstrated in figure 3.4.
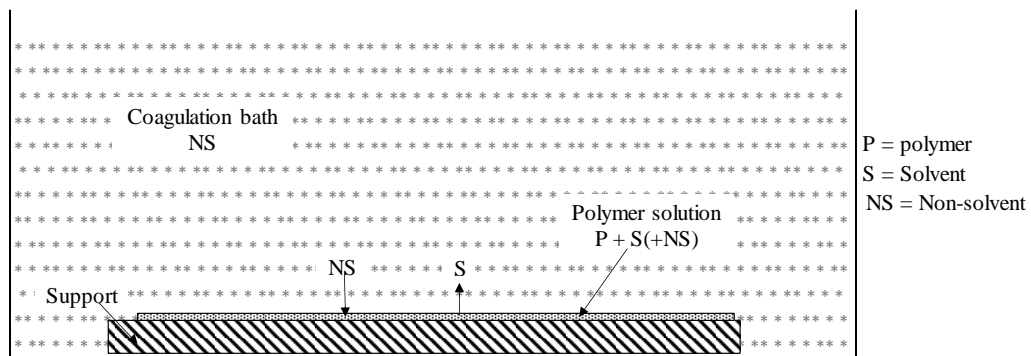
Figure 3.4 Preparation of NF membrane by immersion precipitation. (Graphics altered from: Jye, L. and Ismail, A. 2019).

The last method of preparation is LBL, where an alternating electrostatic absorption of cationic and anionic polyelectrolytes is applied to the surface of a porous supporting membrane. (Jye, L. and Ismail, A 2019). For each step the surface will absorb the polyelectrolyte (PE) and get a charge, and each layer will have alternating charges built up, leading to a PE complex stabilized by strong electrostatic forces, demonstrated in figure 3.5.
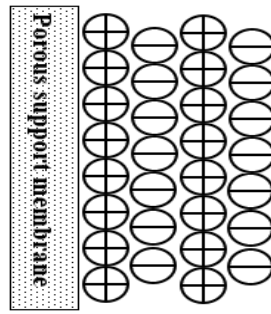
Figure 3.5 Demonstration of the electrostatic structure of LBL NF membranes. (Graphics altered from Jye, L. and Ismail, A 2019)

Two main materials are used for NF membranes in general, polymers and ceramics. Polyethylene, polyvinyl chloride, and polypropylene are generally sought after due to their low cost but are not ideal in conditions that are either high temperature or suscepted to high backwash pressure. Ceramic membranes are generally more thermally, mechanically, and chemically stable (Mulder,M. 1996), however they are more prone to breakage than polymeric membranes. There are many different materials suitable for a ceramic membrane, but the most common ones are aluminium oxide (Al_2O_3), titanium dioxide (TiO_2) and zirconium dioxide (ZrO_2).

There are in general two different modules for application of a NF membrane, the spiral wound (SW) and the hollow-fiber (HF) module (Rackley, S. 2018). A SF module consist of several membranes envelops surrounding a central collecting tube. Each envelope is built up by a feed spacer, the first membrane, a permeate spacer, the second membrane and finally the last feed spacer, sealing the envelope on three sides. This is demonstrated in figure 3.6.
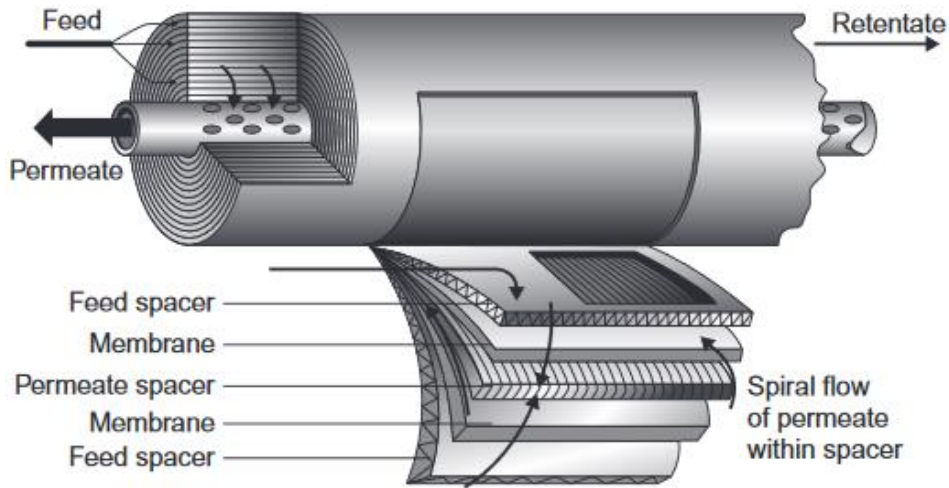
Figure 3.6 Configuration of a spiral-wound membrane. (Graphics from: Rackley.S 2008)

A HF module is built up by stacks of fibres in a closed pressure vessel, where permeate either passes out of the fibres or into them, demonstrated in figure 3.7. A HF module is more mechanically stable and is preferred under high pressure conditions.
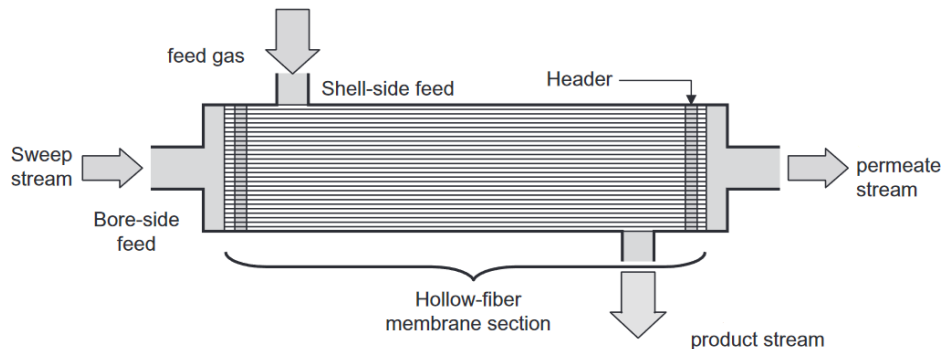
Figure 3.7 Configuration of a hollow fibre module. (Graphics from: Rackley.S. 2008)

Nanofiltration of seawater can give brackish water with different salinity and ion composition based on the choice of membrane material. This makes it an interesting technology for production of intake water for production of post smolt in RAS. As nanofiltration removes divalent ions to a larger extent than monovalent ions, the resulting ion composition is different than what is obtained by mixing seawater and freshwater to a given salinity. Sulfate, being a large and charged ion, can effectively be held back by a NF membrane. By removing sulfate from the intake water, the risk of developing H2S in RAS is lowered. Mixing seawater and freshwater can release toxic metals bound to organic matter in freshwater. This is avoided by producing the intake water with nanofiltration of seawater. In addition, nanofiltration is a good

biological barrier, keeping unwanted bacteria and virus out of the RAS. This makes a NF membrane a viable solution to the H_2S problem. By choosing the right membrane material, synthesis and module, a NF membrane can be designed to remove sulfate and NaCl from saltwater, giving a RAS site a new inlet water that does not need to be mixed with freshwater to meet recommended water quality levels. This includes a sulfate concentration that is lower than that of brackish water.

4 Materials and experimental method

4.1 Setup of nanofiltering membrane at Sunndalsøra

A NF membrane supplied by Akvafresh was installed to desalinate saltwater feed in one RAS system at The Nofima Centre for Sustainable Aquaculture in Sunndalsøra. The membrane was a combination of two different polyamide spiral wound membranes, one with an open structure designed for high sulfate rejection and one with a denser structure aimed lower the final salinity to 12‰. Specific design parameters belong to Akvafresh and will not be disclosed.

Figure 4.1 Picture of the same type of NF membrane installed at Sunndalsøra. (Picture provided by Akvafresh)

From 11.03.30 until 10.06.03, samples were taken from 10 different locations at two different RAS sites. One with the installed NF membrane to desalinate seawater (RAS1), and one where inlet water was a combination of sea water and fresh water that served as a control (RAS2). A process flow diagram for each RAS is given in figure 4.2 and 4.3, with a summary of sample points and descriptions given in table 4.1.

Table 4.1 An overview of each sampling point taken with name, description, and comment.

Sampling point	Name	Decription/Comment
RAS 1		
1	NF,in	Saltwater feed into NF membrane
2	NF,out (1)	Permeate after NF membrane
3	NF,out (2)	Permeate after NF membrane. Sampled to investigate possible trace metals from a metal valve
4	RAS1, Tank(A)	Fish tank sidewall drain 1
5	RAS1, Tank(B)	Fish tank sidewall drain 2
6	RAS1, Sump	Sump
RAS 2		
7	RAS2, Tank(C)	Fish tank sidewall drain 3
8	RAS2, Tank(D)	Fish tank sidewall drain 4
9	RAS2, Sump	Sump
10	FW	Makeup water into biofilter

RAS 1 with membrane
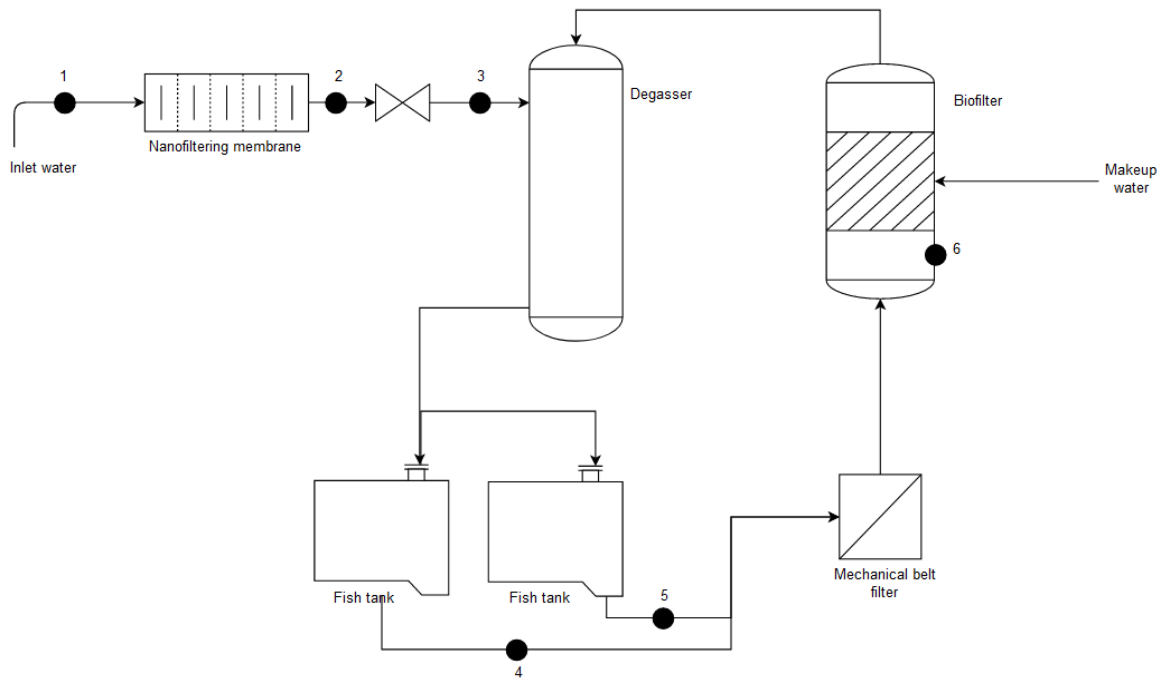
Figure 4.2 Process flow diagram for RAS 1 with sampling points 1-6

RAS 2 without membrane
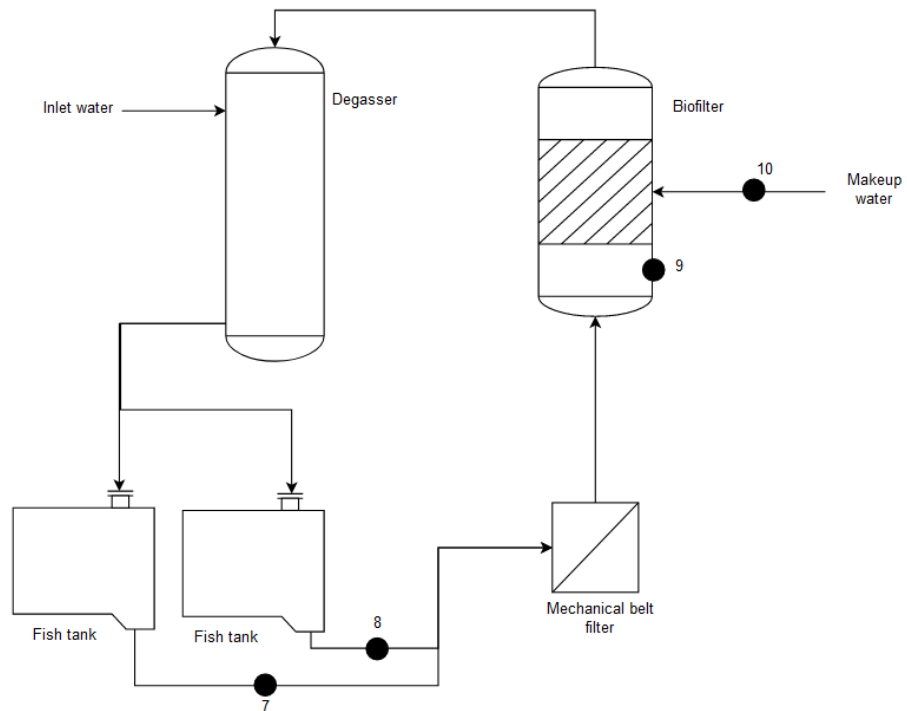
Figure 4.3 Process flow diagram for RAS 2 with sampling points 7-10

4.2 Sampling method

Each component used in the sampling process is listed in table 4.2. Sampling was done by May Britt Mørkedal at The Nofima Centre for Sustainable Aquaculture in Sunndalsøra.

For each sample point a large container was first cleaned with sample water three times, then filled and brought back to the laboratory. Here it was tested for pH, salinity, and conductivity with MultiLine 3620 IDS. Before distribution to centrifugal test tubes, the syringe and test tube were washed 3 times with water from sample point. Then, 15 mL of sample water was extracted with filter attached to the syringe. Filtrated sample water was then used to wash the test tube, making sure to close the lid and shake thoroughly before emptying the test tube. After washing the filter was detached and kept clean, and 30 mL sample water was taken. The filter was then reapplied, and 10 mL sample water was filtrated to two centrifugal test tubes, where one test tube received 3 drops of concentrated nitric acid. Test tubes treated with nitric acid where later used in ICP-MS analysis, and test tubes without were used in IC analysis. Lastly, another 30 mL sample water was extracted with the syringe and transferred to a glass test tube. These samples were used in UV-Vis analysis.

Table 4.2 Overview of equipment used when sampling from RAS at The Nofima Centre for Sustainable Aquaculture in Sunndalsøra.

Equipment	
Syringe	Dispisable, two-piece without needle, PP barrel and PE piston. Product number WVR 613-2033
Syringe filter	PES membrane, 25 mm diameter, 0,2 μm pore size. Product number vWR 514-0074
Centrifugal test tube	Polypropylene, metal free, 15 mL.
Glass test tube	Prewashed and sertified, 40 mL borosilicate, polypropylene/PTFE/Silicone septa. cork. Phoenix product number 9-102-3
Concentrated nitric acid	Ultra pure grad; *Pro Analyses* destilled with Milestone SubPure

4.3 ICP-MS

Inductively coupled plasma mass spectrometry (ICP-MS) is a combination of an inductively couple's plasma (ICP) spectrometer and a mass spectrometer (MS), capable of detecting elements at a very low concentration below ppt (SeQuant 2007). The ICP converts the atom of the element into 2 ions, which are then detected by the MS. In short, the plasma is produced by heating argon gass with a radiofrequency generating coil to a high temperature (6000K – 10000K). The plasma decomposes the sample into 2 ions, which are then extracted and lead into the MS which separates the ions based on their mass/charge ratio (m/z). The intensity of the signal is directly proportional to the concentration of the sample element.

Determination of elemental composition was done by ICP-MS in two batches. The first was conducted by Syverin Lierhagen, with samples from 11.03.20 to 15.04.20 using Agilent 8900 triple quadrupole ICP-MS. The second was conducted by Anica Simic, with samples from 22.04.20 to 10.06.20 using Agilent 8800 triple quadrupole ICP-MS. In total, 27 elements were analyzed, with 7 elements chosen for further investigation by this thesis. The elements included are sulfur, magnesium, potassium, calcium, bromide, silicon, and strontium. Sulfur was chosen as it is the main point of investigation for this thesis. Magnesium, potassium, and calcium were selected as they play an important part in fish metabolism. High concentrations of chloride can interfere with ICP-MS and was therefore not analyzed. Bromide shares similar qualities and was chosen to give some insight to nanofiltration effects on chloride. Silicone was chosen due to the addition of silica compounds in RAS2 to reduce aluminum concentration. Lastly, strontium was chosen due to it being a major seawater ion.

4.4 IC

Ion chromatography separates mixtures into their component ions (Worden, R. 2005). This separation takes place on polymers with columns, where choice of column has a major impact on the results. Before getting injected into the main column, the mixture will pass through a guard column that contains similar materials as the main one. For separation to occur the mixture component needs to exist in a stationary phase and a mobile phase, where the mobile phase is the ionic solution that travel through the system at a constant rate and pressure. Since the mobile phase is conductive, a suppressor is needed to better see the response of low-level components, by removing high conductivity ions from the mobile phase and replacing them with low conductivity ions. To create a stable mobile phase, it is important to utilize an eluent

generator for consistent transportation of sample ions. A representation of the IC system is given in figure 4.4.
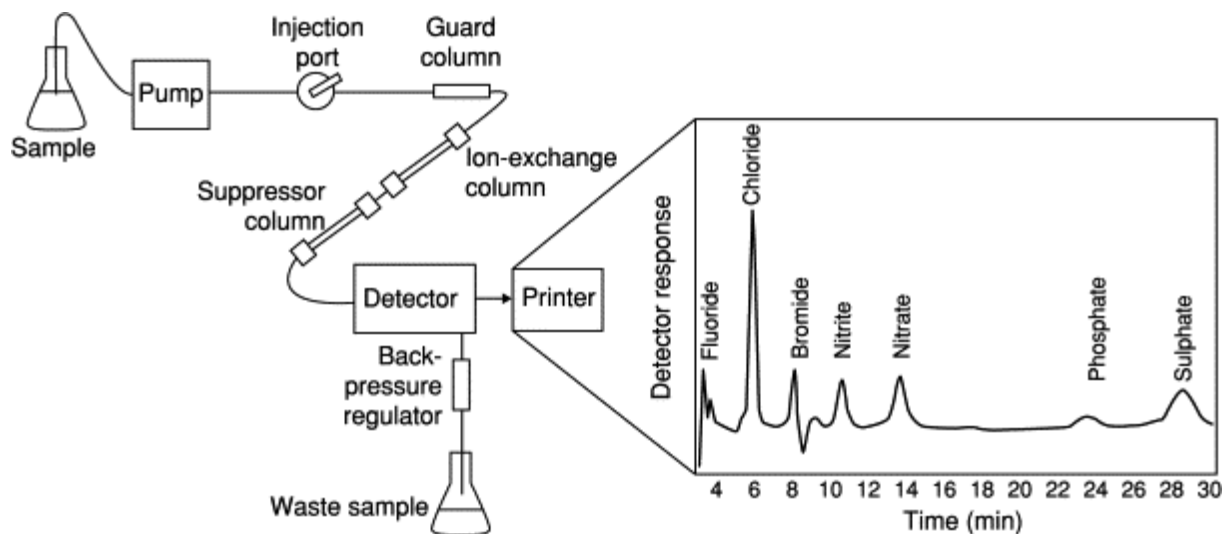
Figure 4.4 Essential components found in an ion chromatograph with a typical output graph. (Graphics from Worden, R. 2005)

Analysis by IC was done with a Methrom 940 Professional IC Vario, with a Metrohm Metrosep A Supp 7 – 250/4.0 column and 3,5 mM Na_2CO_3 used as anion eluent. Analysis was done at 45ºC at a 0,700 mL/min flow, with certified calibration standards given in table 4.2. Samples from RAS1 was diluted 1:10, and RAS2 was diluted 1:50 due to high concentration of chlorine.

Table 4.3 An overview of standard solutions used to make a calibration curve for IC with product number listed for each ion.

IC Standards	
Ion	**Product number**
Fluoride	77365-100 mL
Bromide	43147-100 mL
Nitrate	74246-100 mL
Nitrite	67276-100 mL
Phosphate	38364-100 mL
Sulfate	90071-100 mL

4.5 UV-Vis

Ultraviolet-visible spectroscopy (UV-vis) is the observation of the absorption of electromagnetic radiation in the UV and visible regions of the spectrum. (Atkins,P. 2010 p.228-229). It measured intensity of light passing through a sample (I) and compares it to the intensity of no sample (I_0). The ratio between these two (I/I_0) is called transmittance, and from transmittance you can calculate absorption (A) as -log(T). There is a linear relationship of light absorbed (A) and the concentration of substance of the sample (d), called the Lambert-Beer-Law, where absorbance is given in equation 4.1.

$$A = \varepsilon * c * d \qquad (4.1)$$

Where ε is the molar absorption coefficient ($M^{-1}cm^{-1}$) unique for each sample and d is the optical path length (cm) of cuvette used in analysis. UV-vis can be a useful tool for measuring a number of organic compounds in a given sample. An increase of compounds with aromatic rings, hydroxyl groups or carboxylic acid, as well as an increase of conjugate double bonds will have increased absorption.

Shimadzu UV mini 1240 was used to measure absorbance at 254 nm with a 1cm quartz cuvette. The cuvette was washed prior to sample measurement with Milli-Q water, followed by a wash with sample solution to be analyzed.

4.6 Statistics

4.6.1 Averages

To better compare treated water (RAS1) to control (RAS2), averages were made from 3 different sample points from each system, where RAS1 is given in equation 4.2 and RAS2 is given in equation 4.3.

$$RAS1 = \frac{RAS1,Tank(A)+RAS1,Tank(B)+RAS1,Sump}{3} \qquad (4.2)$$

$$RAS1 = \frac{RAS2,Tank(C)+RAS2,Tank(D)+RAS2,Sump}{3} \qquad (4.3)$$

4.6.2 Standard deviation

Standard deviation was calculated using equation 4.4, to better describe the variation of the dataset.

$$\sigma = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(X_i - \bar{X})^2} \qquad (4.4)$$

where σ is the standard deviation, n is the number of samples and X is the sample value.

4.6.3 Mann-Whitney-U test

The Mann-Whitney-U test is a version of the independent samples t-Test, that can be performed on ranked or ordinal data (Ruxton). This test will compare the mean of the two populations, and the null hypothesis states that the probability is 50% that a randomly drawn member from the first population will have a higher rank than a member from the other population. This is done by first assigning each value a rank based on value. The highest value is rank 1, the second highest rank 2 and so on. U value is then calculated from equation 4.5.

$$U = \sum_{i=1}^{n}\sum_{j=1}^{m} S(X_i Y_i) \qquad (4.5)$$

Where S is 1 if Y<X, ½ if Y=X and 0 if Y>x. Calculated U is then compared to critical value, and null hypothesis is rejected if U<U$_{crit}$.

Assumptions made prior to performing Mann-Whitney-U are as follows:

1. Samples are of one dependent variable measured at the continuous level.
2. Samples consists of two categorical, independent groups
3. There is no relationship between observed effect between the groups
4. Samples are not normally distributed
5. The overall distribution of the samples follows the same general shape

5 Results and discussion

This chapter summarizes and presents the results from all conducted experiments, to investigate the effect of the nanofiltration membrane. Investigations on the relationship between feed and permeate and between treated water and control was done for each parameter. A summary of the results is given in table 5.1. Each parameter will be discussed further in their own separate section. Sample points discussed in this chapter are *NF,in* (feed), *NF,out* (permeate), *RAS1*(treated water) and *RAS2*(control).

Table 5.1 Summary of each measured parameter with calculated difference presented in associated units and in percentage, between feed and permeate and between treated water and control.

Parameter	Feed	Permeate	Difference	% Mean	RAS1	RAS2	Difference
pH	7,940	8,060	0,120	1,511 %	7,71	7,680	-0,030
Salinity (‰)	32,210	12,840	-19,370	-60,137 %	12,07	11,970	-0,100
Conductivity (mS/cm)	49,800	21,500	-28,300	-56,827 %	12,07	20,390	8,320
S (mg/L)	857,310	56,850	-800,460	-93,369 %	26,55	308,500	281,950
$SO_4^{(2-)}$ (mg/L)	-	-			30,14	212,980	182,840
Mg+ (mg/L)	119,980	338,500	218,520	182,130 %	269,68	293,980	24,300
Ca2+ (mg/L)	398,550	126,400	-272,150	-68,285 %	95,93	141,200	45,270
K+ (mg/L)	318,670	157,990	-160,680	-50,422 %	125,29	118,250	-7,040
Br- (mg/L)	69,520	30,580	-38,940	-56,013 %	27,13	23,970	-3,160
Si4+ (mg/L)	0,160	0,100	-0,060	-37,500 %	0,14	4,300	4,160
Sr- (mg/L)	7,375	2,170	-5,205	-70,576 %	0,169	2,568	2,399
Absorbance	0,023	0,017	-0,006	-26,087 %	0,051	0,049	-0,002

5.1 The effects of nanofiltering membrane on pH, salinity, and conductivity

Descriptive values for pH, salinity and conductivity are given in table 5.2, with results from test of significance given in table 5.3 and all measured values are given in appendix A.1. Comparing permeate and feed there is a significant difference in all three parameters. However, this is not the case when comparing treated water with control, where none of the parameters was proven to be significantly different. Therefore, this section is further divided to discuss each individual parameter.

Table 5.2 Descriptive values of pH, salinity and conductivity for *NF,in*, *NF,out*, *RAS1* and *RAS2*

	Sample point	Average	Median	Min	Max	Standard deviation
pH	NF, in	7,945	7,938	7,872	8,046	0,043
	NF, out	8,057	8,061	7,918	8,131	0,052
	RAS1	7,706	7,680	7,373	7,942	0,150
	RAS2	7,680	7,706	7,275	7,876	0,124
Salinity [%]	NF, in	32,208	33,800	12,900	34,200	5,579
	NF, out	12,842	12,000	10,300	33,800	4,236
	RAS1	12,067	12,100	11,300	12,800	0,383
	RAS2	11,972	12,000	11,300	12,700	0,386
Conductivity [mS/cm]	NF, in	49,800	52,250	21,800	53,000	8,450
	NF, out	21,497	20,500	13,330	52,200	6,623
	RAS1	12,067	20,450	0,021	21,600	3,334
	RAS2	20,387	20,350	19,270	21,500	0,610

Table 5.3 An investigation to determine if there was a significant difference of pH, salinity or conductivity before and after the nanofilter (*NF,in/NF,out*) and between treated and untreated water in RAS phase (*RAS1/RAS2*). A Mann-Whitney-U test with null hypothesis H_o: $P(x_i>y_j) = 0,5$ versus alternative hypothesis H_1: $P(x_i>y_j) \neq 0,5$ was used.

Parameter	Sample point	Mann-whitney U	Z-value	P-value	Reject Ho
pH	Nf,in/Nf,out	17,000	-3,463	0,001	Yes
	RAS1/RAS2	689,500	-0,710	0,478	No
Salnity	Nf,in/Nf,out	8,000	-3,937	0,000	Yes
	RAS1/RAS2	640,500	-1,208	0,227	No
Conductivity	Nf,in/Nf,out	7,000	-3,767	0,000	Yes
	RAS1/RAS2	661,000	-0,636	0,525	No

5.1.1 pH

Figure 5.1 demonstrates the differences in pH for feed, permeate, treated water and control. A box plot was generated to inspect the distribution further. Data shows a difference in averages of feed and permeate of 0,112. It is a small but significant increase and puts pH above recommended levels of 6,2-7,8. One possible reason for this increase could be the nanofilter retained a small amount of bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}). As discussed earlier in section 3, the design of the nanofilter can allow for a partial diffusion of some multivalent ions, depending on size on charge. This small increase is only temporary, as there was no significant difference in pH between treated water and control throughout the experiment.
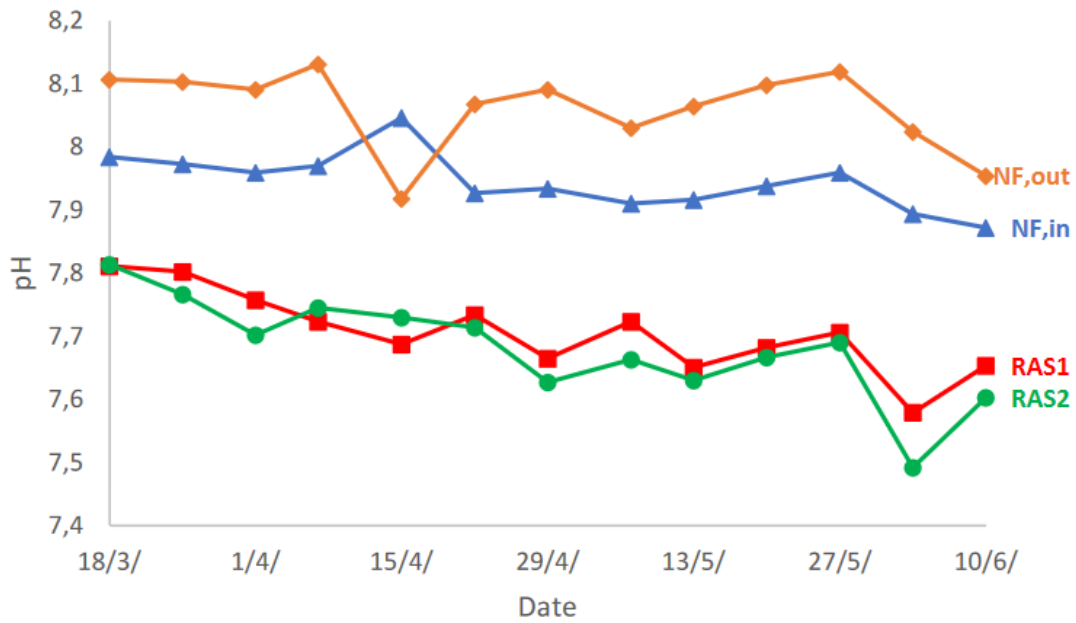
Figure 5.1 Measured pH of feed(*NF,in*), permeate(*NF,out*), treated water(*RAS1*) and control(*RAS2*) plotted for each sampling date.
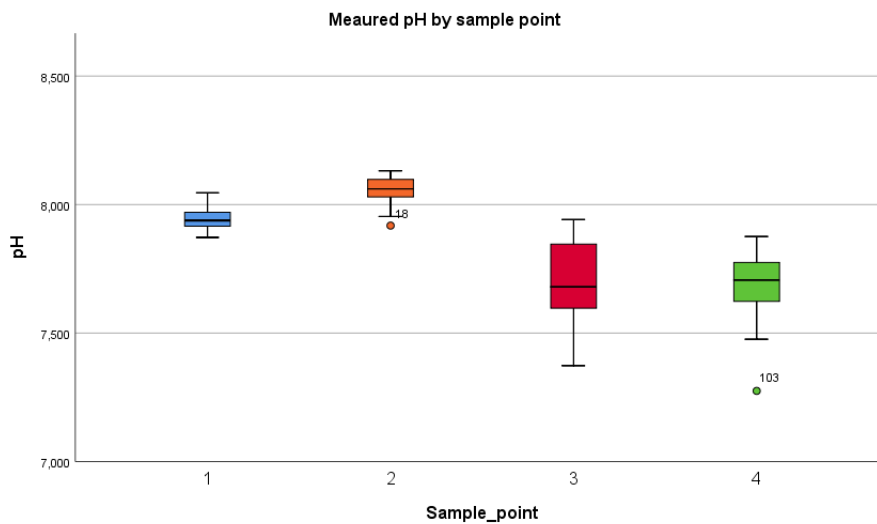
Figure 5.2 Box plot of measured pH, where sample points 1-4 represents feed(*NF,in*), permeate(*NF,out*), treated water(*RAS1*) and control(*RAS2*) respectively.

5.1.2 Salinity and conductivity

Figure 5.3 and 5.4 demonstrates the differences in salinity and conductivity for feed, permeate, treated water and control. Box plots were generated to inspect the distribution further. As with pH, there was a significant difference between both salinity and conductivity levels of feed compared to permeate. This is to be expected, as the nanofilter can be designed to retain ions of certain size and charge. A decrease in salinity points to a decrease in ions such as Mg^{2+}, Ca^{2+}, K^+ and SO_4^{2-}, which would also decrease conductivity due to the lack of charged ions. This will be investigated further in the results from the IC-PMS and IC analysis. When comparing treated water to control there is no significant difference, which means the treated water will achieve the same water quality standard as the currently used brackish water.
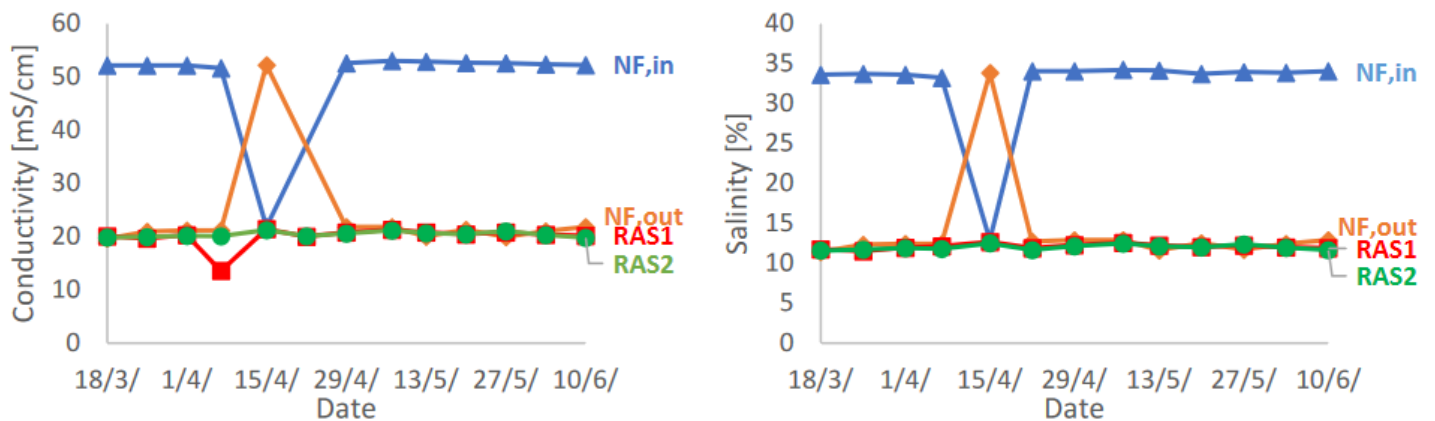
Figure 5.3 and 5.4 Measured conductivity (left) and salinity (right) for feed(*NF,in*), permeate(*NF,out*), treated water(*RAS1*) and control(*RAS2*) plotted for each sampling date.
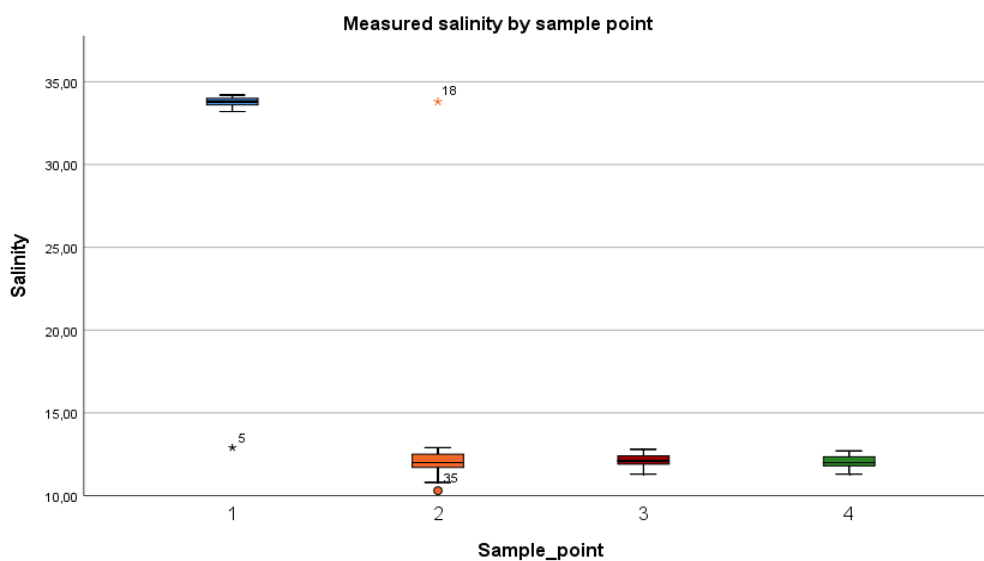
Figure 5.5 Box plot of salinity of feed(*1*), permeate(*2*), treated water(*3*) and control(*4*).
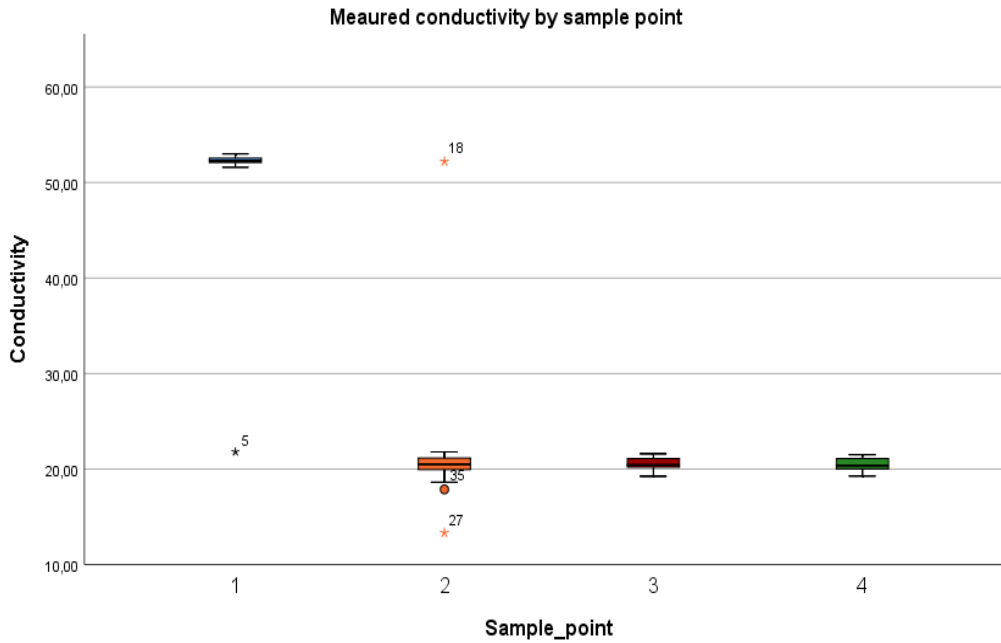
Figure 5.6 Box plot of conductivity of feed(*1*), permeate(*2*), treated water(*3*) and control(*4*).

5.2 Effect of nanofiltering membrane on elemental composition

Determination of elemental composition was done by ICP-MS. In total, 27 elements were analyzed, with 7 elements chosen for further investigation by this thesis. The elements included are sulfur, magnesium, potassium, calcium, bromide, silicon, and strontium.

5.2.1 Efficiency on sulfur removal measured by ICP-MS and IC

Reduction of sulfur was the main point of interest. As discussed in section 2.1.3, reducing total sulfur content will limit accumulation of harmful H_2S, and was one of the key design aspects of the nanofiltering membrane. Elemental composition data from ICP-MS analysis is visualized in figure 5.7 with data given in appendix A.2, together a generated box plot shown in figures 5.8 with accompanying descriptive values given in table 5.4 and statistical parameters given in table 5.5. In both point of comparison there was a significant decrease in sulfur, with a 93% reduction of average from feed to permeate, and a 91% reduction between the average of treated water and control, implying that the specific nanofilter design is working as intended. When observing the box plots, there are two points of interest. *NF,in* and *NF,out* again seem to line up better with their counterparts, observable both in the figure and at outliers in the box plot.

There is also a high standard deviation for *NF,in*, *NF,out* and RAS2. Measured concentration of sulfur is not approximating a linear trend, having higher values between 22.04.20 before it evens out after 27.05.20. It follows that all subsequent sample points experience the same rise in value, since they are all part of an almost closed loop system with *NF,in* as inlet water.
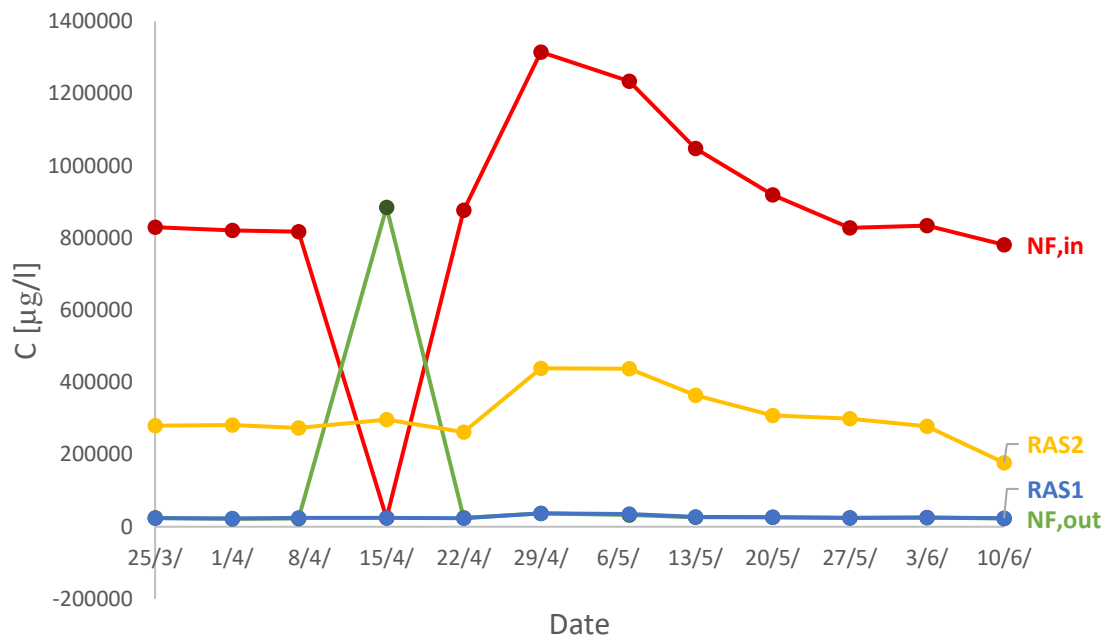
Figure 5.7 Measured sulfur concentration [µg/l] for *NF,in*, *NF,out*, *RAS1* and *RAS2* from 11.03.20 until 10.06.20, found by ICP-MS.

Table 5.4 Descriptive values of elemental sulfur concentration determined by ICP-MS [µ/L] for *NF,in*, *NF,out*, *RAS1* and *RAS2*.

Sample point	Average	Median	Min	Max	Standard deviation
NF, in	857 313,26	831 792,81	24 369,73	1 314 804,22	280 015,85
NF, out	56 851,39	23 473,44	19 432,89	884 867,28	162 437,51
RAS1	26 554,83	24 586,29	16 859,89	39 823,53	5 363,53
RAS2	308 498,41	282 085,68	252 342,05	460 536,91	61 581,16

Table 5.5 An investigation to determine if there was a significant difference in concentration of elemental sulfur before and after the nanofilter (*NF,in/NF,out*) and between treated and untreated water in RAS phase (*RAS1/RAS2*). A Mann-Whitney-U test with null hypothesis H_o: $P(x_i>y_j) = 0,5$ versus alternative hypothesis H_1: $P(x_i>y_j) \neq 0,5$ was used.

| Sample point	Mann-whitney U	Z-value	P-value	Reject Ho
Nf,in/Nf,out	16,000	-3,768	0,000	Yes
RAS1/RAS2	16,000	-7,842	0,000	Yes
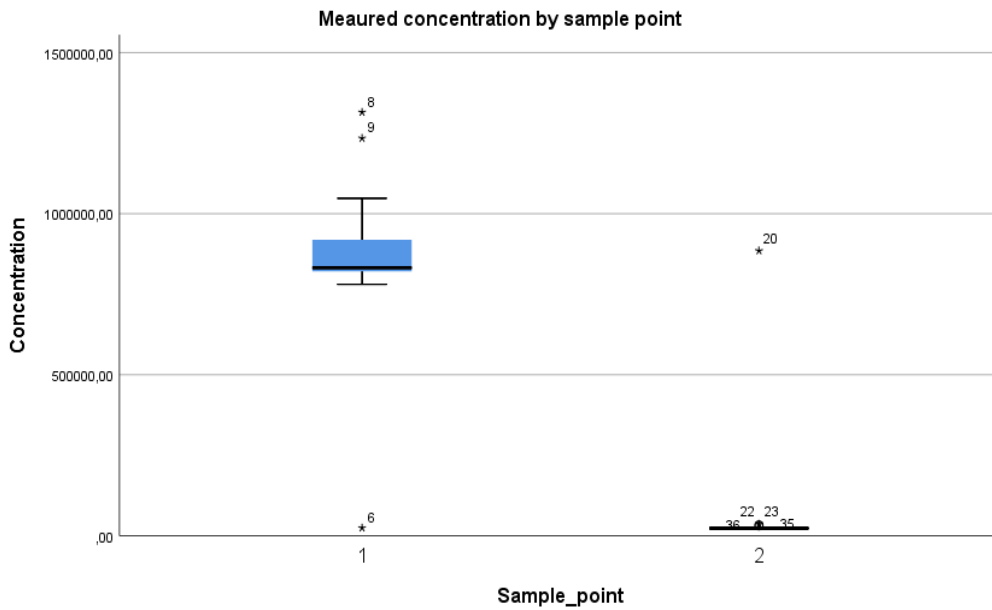
Figure 5.8 Box plot of elemental sulfur concentration of feed(*1*) and permeate(*2*)
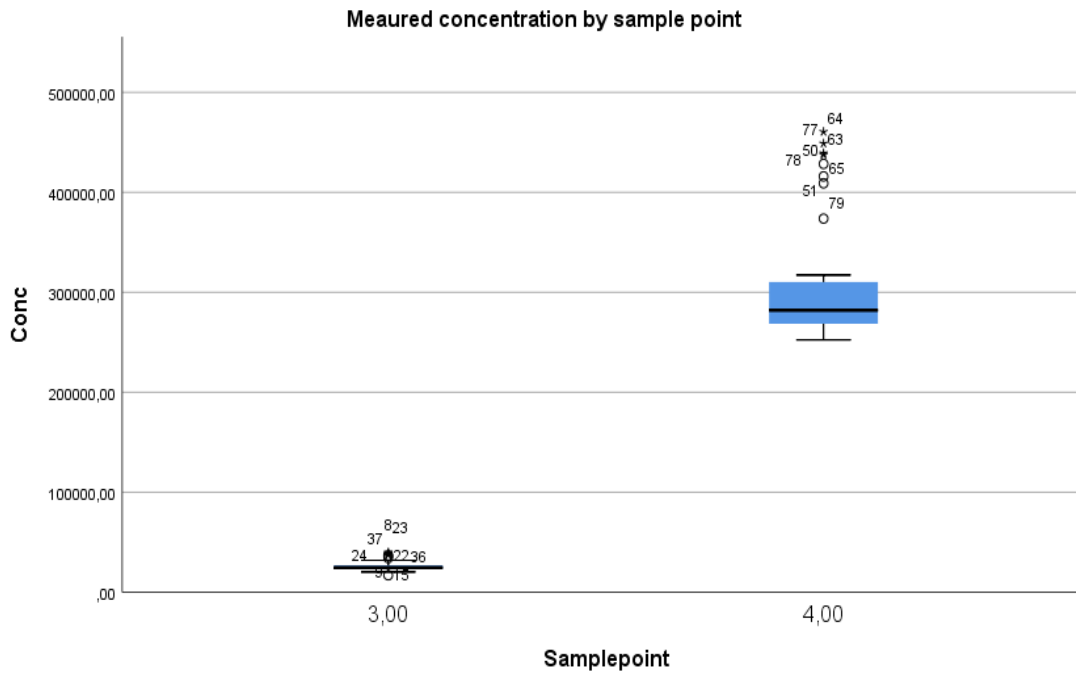
Figure 5.9 Box plot of elemental sulfur
concentration of treated water(*3*) and control(*4*).

As discussed in section 2.1.3 sulfur found in seawater is present as different compounds. An IC analysis was done to investigate the percentage of sulfur compound present as sulfate. Results from this analysis is represented graphically in figure 5.10 with values in appendix A.3 where measured sulfate has been divided by 3 to convert to sulfur mass (Fang et al. 2015) and compared to ICP-MS data. A box plot was generated to further investigate distribution of data, with descriptive statistical values given in table 5.6. To further investigate the relationships between IC and ICP-MS data, tests of significance were performed on three levels; (1) Between *RAS1* and *RAS2* measured by IC, (2) between *RAS1* measured by IC and ICP-MS and (3) between *RAS2* measured by IC and ICP-MS, with values in table 5.7.

Results from IC analysis was highly volatile, with a range of 447,29 mg/L for *RAS1* and 2385 mg/L for *RAS2*. High concentration of chloride is known to be challenging in IC analysis (Anes et al. 2019) and may have interfered with the IC quantification of sulfate. This highly suggests that there was an issue with dilution, where samples were not diluted to the extent required to limit interference. There is however an observable trend, where there is a significant difference between *RAS1* and *RAS2*. This mirrors the results from the ICP-MS analysis, providing further support that nanofiltration was efficient at removing sulfate.

It is expected that the elemental sulfur found in the samples is mainly sulfate. As discussed in section 2.1.3 H_2S will only occur in the anaerobic layer of the biofilm if it has access to sulfate, and with the overall sulfate reduction the connected H_2S concentration can also be expected to be low. This would be further proved by comparing elemental concentration to ion concentration, but there was found no significant difference between the two and therefore no definitive conclusion can be made on H_2S concentration in the RAS site.
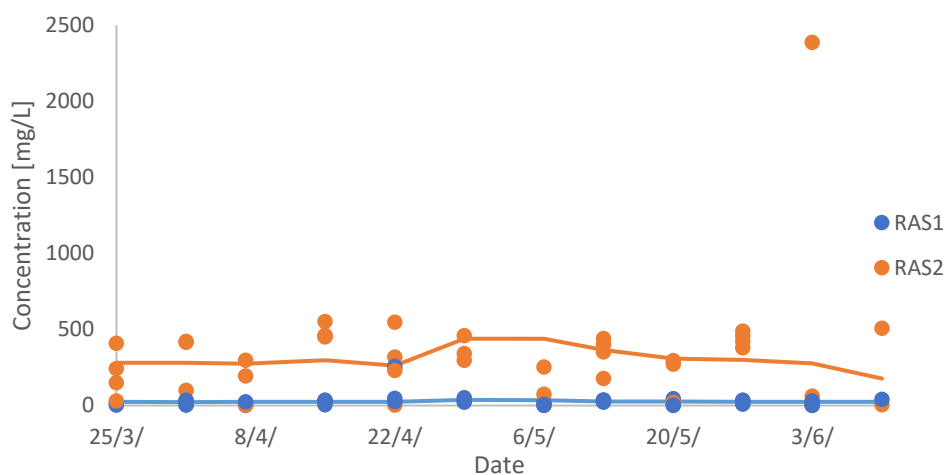
Figure 5.10 Concentration of sulfate measured by IC and converted to sulfur for each sample date, compared to ICP-MS measured sulfur concentration represented as lines.

Table 5.6 Descriptive values of elemental sulfur concentration [mg/L] for *RAS1* and *RAS2* measured by IC.

Sample point	Average	Median	Min	Max	Standard deviation
RAS1	28,65	25,21	0,64	258,13	39,61
RAS2	317,88	297,59	0,14	2 385,47	364,88

Table 5.7 An investigation to determine if there was a significant difference in concentration of sulfur between treated and untreated water in RAS phase (*RAS1/RAS2*) measured by IC, and if there was a significant difference between sulfur measured by ICP-MS and sulfur measured by IC. A Mann-

Whitney-U test with null hypothesis H_o: $P(x_i>y_j) = 0,5$ versus alternative hypothesis H_1: $P(x_i>y_j) \neq$ 0,5 was used.

Test	Mann-whitney U	Z-value	P-value	Reject Ho
RAS1/RAS2 IC	350,000	-4,962	0,000	Yes
RAS1/RAS1 IC/ICP-MS	794,000	-1,127	0,260	No
RAS2/RAS2 IC/ICPMS	909,500	-0,130	0,897	No
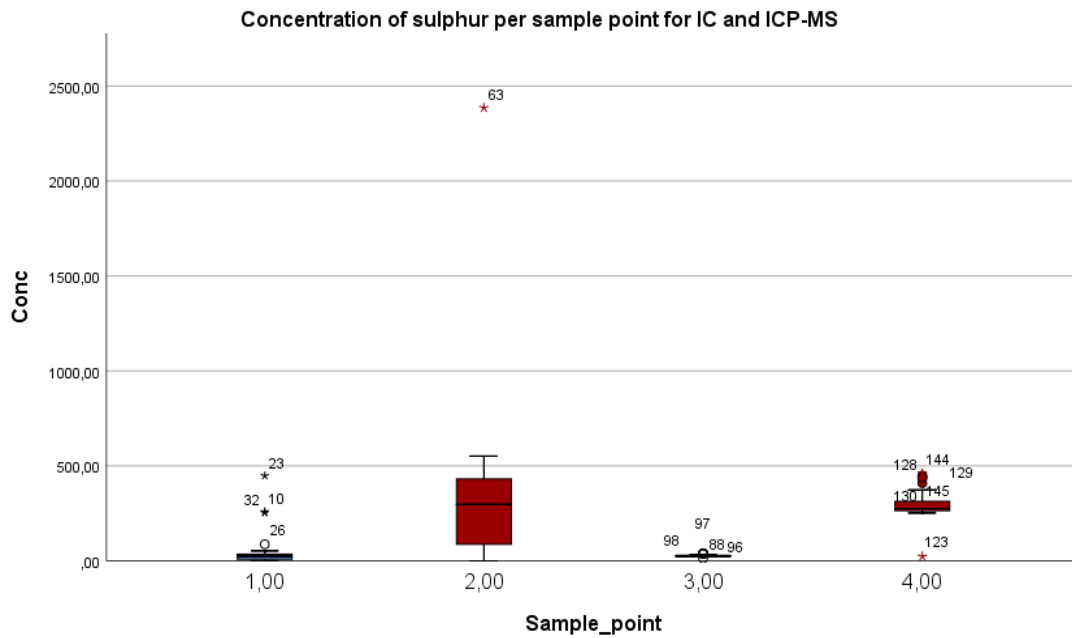
Figure 5.11 Box plot comparing distribution of sulfur measured by ICP-MS (1,3) and measured by IC (2,4)

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5.2.2 Other ions of importance

Results from ICP-MS analysis for magnesium, silicone, potassium, calcium, bromide, and strontium is given in figure 5.12, with a summary of important calculated data given in table 5.7.
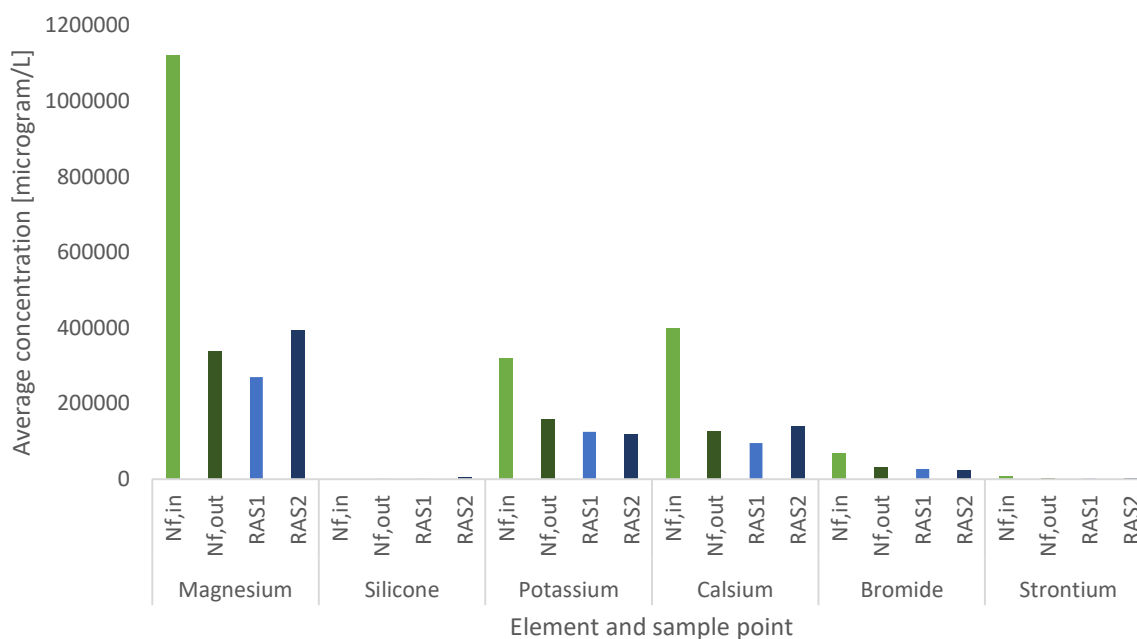
Figure 5.12 A comparison of average elemental concentration for magnesium, silicone, potassium, calsium, bromide and strontium for each sample point

Table 5.8 Calculated differences of Mg, Si, K, Ca, Br and SR between NFin/NFout and RAS1/RAS2, with significance.

Element	Averages [microgram/L]							
	Nf,in	Nf,out	Diff	p	RAS1	RAS2	Diff	p
Mg	1 119 975,385	338 495,357	69,78 %	0,000	269 680,829	392 977,279	-45,72 %	0,000
Si	156,571	96,214	38,55 %	0,006	137,046	4 304,506	-3040,91 %	0,000
K	318 667,237	157 989,286	50,42 %	0,011	125 286,404	118 249,977	5,62 %	0,053
Ca	398 551,093	126 398,362	68,29 %	0,000	95 932,881	141 204,582	-47,19 %	0,000
Br	69 519,866	30 583,869	56,01 %	0,000	27 128,016	23 966,663	11,65 %	0,000
Sr	7 374,622	2 170,228	70,57 %	0,000	1 689,781	2 568,321	-51,99 %	0,000

Before nanofilter, magnesium levels were found to be the highest out of the selected ions, and amount decreased in the order of Ca, K, Br, Sr, and Si respectively. This fits with expected values presented in section 2.1.2. All ions investigated had a significant decrease after nanofiltration.

When comparing treated water to control a few things are apparent. There is a higher concentration of magnesium, silicone, calcium, and strontium in control, where inlet water is a mixture between saltwater and seawater. As discussed earlier, Mg, Ca and Sr are all major ions of seawater, and to achieve lower concentrations of these ions require further dilution with freshwater, which might not be ideal when considering other water quality parameters. Silicon is much higher in RAS2 than in RAS1. When looking at data from Appendix A.2, freshwater (FW) samples have a very high concentration of silicon compared to other sampling points. This is caused by the addition of silica-compounds to inlet freshwater to reduce the harmful effects of aluminum compounds.

Bromine and potassium had higher concentrations in RAS 1. Increase in potassium was not significant, and it is likely that treated water will have the same concentration of potassium as untreated water. A higher bromine concentration is caused by filtration, where the nanofilter did not reduce bromine enough to be equal to that of brackish water. This could however provide some insight to overall concentration of chlorine. Chlorine was excluded from analysis by ICP-MS, due to the high concentration it has in seawater and the potential to cause interference with other measurements, but since chlorine and bromine share similar qualities as monovalent ions, it can be assumed that the NF membrane will reduce Cl to a similar extent.

The effect of this change in ion composition was not studied, therefore it is not known what kind of effect this will have on fish welfare. As discussed in section 2.1.2, magnesium, calcium and potassium all play important parts in fish metabolism. Since magnesium and calcium had an overall decrease this could effect the overall fitness of the fish, but it is unknown if this effect would prove to be harmful over time. There was found an increase of potassium almost at a 5% level of significance, but here it is also unknown if this effect would prove to be either beneficial or harmful to the fish.

5.3 UV-vis

UV-vis analysis was performed to investigate if nanofiltration had an impact on accumulation of organic matter. Measured values and calculations are given in appendix A.4.

There was an average reduction of 28% percent absorbance in desalinated water, indicating that larger organic compounds did not permeate the membrane. No significant difference was found between RAS1 and RAS2. Data from test of significance is given in table 5.8. There was an overall increase in absorbance for all datapoints, demonstrated in figure 5.13
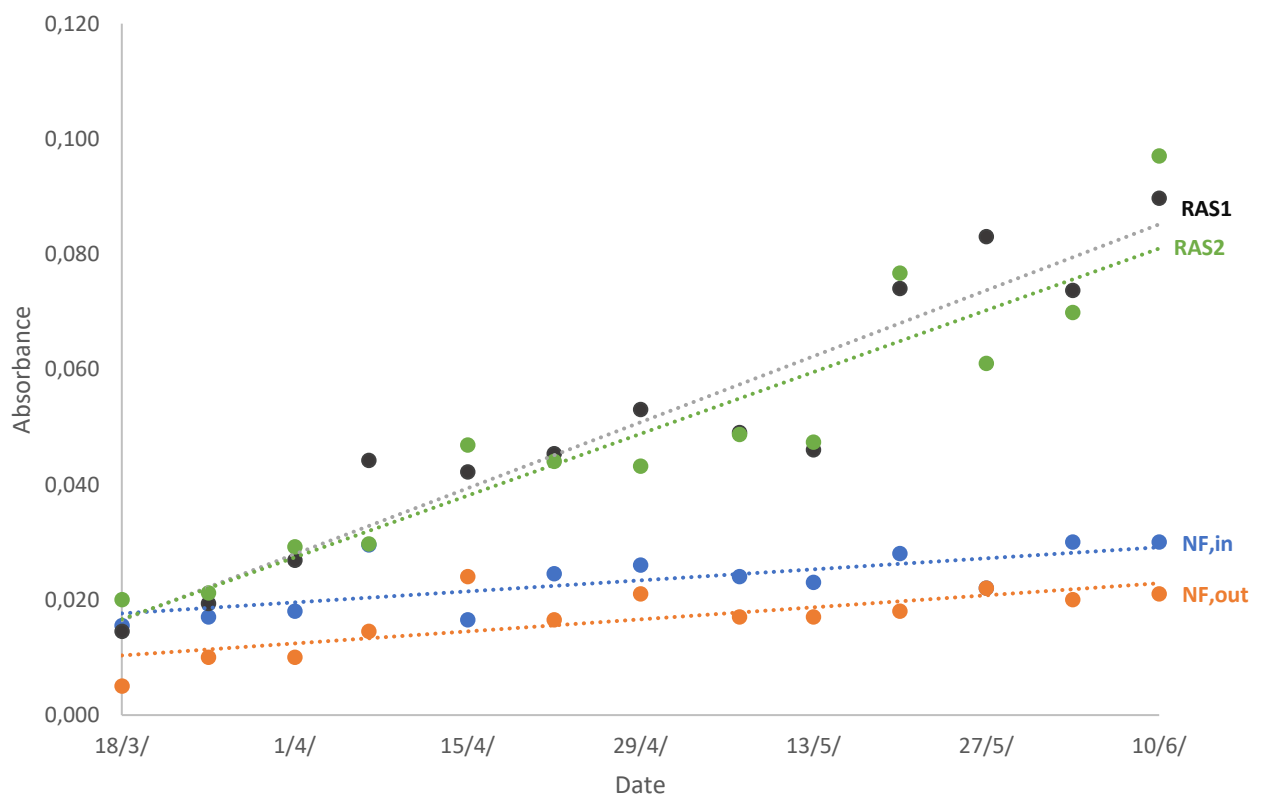
Figure 5.13 Measured absorbance plottet against each date for NF,in, NF,out, RAS1 and RAS2.

All points of measurement has an linear increase in absorbance over time, a smaller one for NF,in and NF,out, and a quite substantial one for RAS1 and RAS2. In total, 130 samples were analyzed using the same quartz cuvette, and contamination seems likely to be the cause of this increase, but this would not explain the difference in rate of increase between all four sample points. If contamination was the only issue, one would expect that increase of absorption would happen at the same rate for all sample points. However, RAS1 and RAS2 increase at a much

higher rate. One possible explanation of this is fish waste. Throughout the experiment salmon lived in the tanks, consuming feed, and excreting waste. Over time it is expected that particle waste in fish tanks will increase overall, making it likely that the steady increase of absorbance is due to fish waste and uneaten fish food.

Table 5.9 Descriptive values of measured absorbance for NF,in, NF,out, RAS1 and RAS2

Sample point	Average	Median	Min	Max	Standard deviation
NF, in	0,023	0,024	0,016	0,030	0,005
NF, out	0,017	0,017	0,005	0,022	0,005
RAS1	0,051	0,049	0,015	0,090	0,024
RAS2	0,049	0,050	0,020	0,097	0,022

Table 5.10 An investigation to determine if there was a significant difference in absorption between treated and untreated water in RAS phase (*RAS1/RAS2*) measured by UV-VIS. A Mann-Whitney-U test with null hypothesis H_o: $P(x_i > y_j) = 0,5$ versus alternative hypothesis H_1: $P(x_i > y_j) \neq 0,5$ was used.

Sample point	Mann-whitney U	Z-value	P-value	Reject Ho
Nf,in/Nf,out	33,000	-2,646	0,007	Yes
RAS1/RAS2	701,000	-0,595	0,552	No

5.3.1 Outliers

At 15.04.20 there is two points of interest, where it appears that values of *NF,in* and *NF,out* has a better fit within their counterpart's dataset than within their own. This trend of outliers is consistent in all points of measurements, making it likely that an error with labelling of samples has occurred. The outliers will therefore have an effect on statistical parameters, making it likely that means, medians, minimum values, maximum values and standard deviations are skewed for *NF,in* and *NF,out* and that measured significance between the two sample points is somewhat lower.

5.4 Further work

This thesis could only focus on a select few ions, and there are many elements left to investigate. Since there was a difference in elemental composition between the treated water and the untreated water, it would be interesting to see what kind of long term effects this would have on fish welfare, growth and survivability.

More testing is needed with IC. Current dataset does not give a conclusive answer to the relationship between sulfate and elemental sulfur measured by ICP-MS and would be an important parameter to figure out for future investigation on sulfurs behavior in RAS. For a future analysis of samples with IC, a higher dilution is recommended.

There is a method that directly measures H_2S concentration (Langeteig, S. 2019), by utilizing the Diffusive Gradients in Thin films (DGT) instrument. Access to directly measured H_2S would provide a great benefit into the investigation on desalination of water, by giving a more detailed and accurate description of the sulfur equilibrium.

Since this thesis only performed a UV-Vis analysis, there can only be made general assumption on the concentration of organic compounds. A future research with a TOC and DOC analysis could be interesting, as it could give a more detailed picture on the specific parameters of the nanofiltering membrane and its effect on different organic compounds.

It will be interesting going forward what will be the optimal solution to the H_2S problem. This thesis has not taken cost into consideration, and there is therefore no final conclusion to be made on what is the optimal solution. Using a nanofiltering membrane has been proved to be an adequate alternative, but there is yet to be done a cost analysis comparing nanofiltration to optimizing tank design or adding nitrate to keep a healthy biofilm.

6 Conclusion

Desalination of saltwater proved to be an effective method for removal of sulfate, removing 93% of all sulfur compounds. When comparing treated water against control, treated water had on average 91 % less sulfur and 86 % less sulfate. In theory, this would lead to a drastic reduction in the amount of H_2S produced making desalination with nanofiltration a very viable alternative in RAS. The nanofiltering membrane did decrease the pH, salinity, and conductivity of the inlet, but there was no significant difference when compared to control, providing further evidence that this treatment method will not have any harmful effects on water quality.

Nanofiltered feed water had a reduction in magnesium, silicone, potassium, calcium, bromide, and strontium in inlet water. When comparing treated water to control there was an increase of bromide, and a reduction of magnesium, silicone, calcium, and strontium. It is not known wether or not this will influence fish welfare, growth, or survivability in the long term.

There was found to be a reduction in organic compounds between feed and permeate water, but no significant difference between treated water and control. The latter two had a steep increase over time compared to the prior two. This is likely due to the natural growth cycle of the fish, where they produce more particle waste as they grow.

References

Aslam, S., Navada, S., Bye, G., Mota, V., Terjesen, B., and Mikkelsen,Ø. *Effects of CO2 on elemental concentrations in recirculating aquaculture system tanks* Aquaculture 511(734254) *https://doi.org/10.1016/j.aquaculture.2019.734254*

Atkins, P. 2010 *Inorganic Chemistry*(5th edition) 2010 Oxford university press ISBN10-0199236178

Attramadal, K.J.K., Øie, G., Størseth, T.R., Alver, M.O., Vadstein, O., and Olsen, Y. (2012a).*The effects of moderate ozonation or high intensity UV-irradiation on the microbial environ-ment in RAS for marine larvae* Aquaculture330-333, p. 121–129

Boyd,C. *Hydrogen Sulfide Toxic, But Manageable,* Global Aquaculture Ad-vocate, vol. 17, no. 2, pp. 34–36, 2014.

Byrne, R. Howard , Mackenzie, . Fred T. and Duxbury, . Alyn C. *Seawater. Encyclopedia Britannica.* Accsessed 18.06.21 from *https://www.britannica.com/science/seawater*

Chiam, C.K. and Sarbatly, R. (2011). "Purification of aquacultural water: conventional and newmembrane-based techniques".Separation and Purification Reviews40.2, pp. 126–160

Clapham,E *Calcium Signaling* 2008 Cell 131(6) p. 1047 *https://doi.org/10.1016/j.cell.2007.11.028*

Droas, M. *Stability and lifespan analysis of nanofiltration membrane in binary solution* 2019 NTNU

El-Mowafi, A. and Maage,A. *Magnesium requirements of Atlantic salmon (Salmo salar L.) parr in seawater-treated fresh water* 1998 Aquaculture nutrition 4(31-38)

Forgan, L. G., & Donald, J. A. (2016). Subchapter 103C - *Hydrogen Sulfide.* Handbook of Hormones San Diego: Academic Press.

Holmer, M. and Storkholm, P. *Sulfate reduction and sulfur cycling in lake sediments.* Freshwater Biology (2001) **46**, 431-451

Iversen, A., Asche, F., Hermansen, Ø., Nystøyl, R. (2020) *Production cost and competitiveness in major salmon farming countries 2003–2018, Aquaculture*, 522(735089) *https://doi.org/10.1016/j.aquaculture.2020.735089.*

Iwama G.K.*,* Thomas P.T.*,* Forsythe R.B. *&* Vijayan M.M. *(1998) Heat shock protein expression in fish. Reviews in Fish Biology and Fisheries 8, 35–56.*

Jye, L. and Ismail, A. *Nanofiltration Membranes* 2019 CRC Press Tyler And Francis Group

Kalantarian,S., Rafiee, G., Farhangi, M., and Mojazi, B. *Effect of Different Levels of Dietary Calcium and Potassium on Growth Indices, Biochemical Composition and Some Whole Body Minerals in Rainbow Trout (Oncorhynchus Mykiss) Fingerlings* 2013 Journal of Aquaculture 4(3) DOI: 10.4172/2155-9546.1000170

King, K., Flick, G., Smith, S., Pierson, M., Boardman, G., and Coale, C. *Response of Bacterial Biofilms in Recirculating Aquaculture Systems to Various Sanitizers* (2008) Journal of Applied Aquaculture 20(2) DOI:10.1080/10454430802191766

Langeteig, S. *Bakgrunnsnivåer av hydrogensulfid I RAS, produksjon av hydrogensulfid fra fiskeslam ved ulike saliniteter, og effekten av å tilsette nitrat* 2019 Master thesis NTNU

Lekang, O *Aquaculture Engineering* 2020 John Wiley and Sons Limited

Lydersen, E., *The Solubility and Hydrolysis of Aqueous Aluminium Hydroxides in Dilute Fresh Waters at Different Temperatures* 1990 Nordic Hydrology 21(195-204)

Ministry of Trade, Industry and Fisheries (2014-2015), Forutsigbar og miljømessig bærekraftig vekst i norsk lakse- og ørretoppdrett. Accessed 16.06.21 from *https://www.regjeringen.no/no/dokumenter/meld.-st.-16-2014-2015/?ch=1*

Molleda, I.; Thorarensen, H.; Johannsson, R.,*Water quality in recirculationaquaculture systems (RAS) for arctic charr (Salvelinus alpinus L.) Culture* 2008 Holar University College Iceland

Mulder, M. 1996. *Basic Principles of Membrane Technology*(2nd edition.). Dordrecht: Kluwer Academic Publishers

Noble, C., Nilsson, J., Stien, L. H., Iversen, M. H., Kolarevic, J. & Gismervik, K. (2018). *Velferdsindikatorer for oppdrettslaks: Hvordan vurdere og dokumentere fiskevelferd.* Nofima Accessed 19.06.21 from *https://nofima.no/wp-content/uploads/2016/06/Velferdsindikatorer-for-oppdrettslaks-2018.pdf*

Norwegian Statistics (29.10.20) Aquaculture. Accessed 16.06.21 from *https://www.ssb.no/jord-skog-jakt-og-fiskeri/statistikker/fiskeoppdrett*

Olaussen, J., (2018) *Environmental problems and regulation in the Aquaculture industry. Insights from Norway.* DOI:10.1016/j.marpol.2018.08.005

Pedersen, P. B., von Ahnen, M., Fernandes, P., Naas, C., Pedersen, L.-F., & Dalsgaard, J. (2017). *Particle surface area and bacterial activity in recirculating aquaculture systems.* Aquacultural Engineering, 78, 18-23. *doi:https://doi.org/10.1016/j.aquaeng.2017.04.005*

Pinnau, I. *Membrane separations* 2000 Encyclopedia of Separation Science p. 1755-1764 https://doi.org/10.1016/B0-12-226770-2/05241-8

Poleo,A., and Hytterød,S. *The effect of aluminium in Atlantic salmon (*Salmo salar*) with special emphasis on alkaline water 2003* Journal of Inorganic Biochemistry 97(1) *https://doi.org/10.1016/S0162-0134(03)00261-7*

Rackley,S. *Membrane separation systems* 2018 Carbon Capture and Storage (2nd edition) Butterworth-Heinemann p. 187-225 *https://doi.org/10.1016/B978-0-12-812041-5.00008-8*

Saleh, T. and Gupta. K *Nanomaterial and Polymer Membranes* 2016 Elsevier Inc. ISBN: 978-0-12-804703-3

Schramm,A., Beer, D., Gieseke,A. and Amann,R. *Microenvironments and distribution of nitrifying bacteria in a membrane-bound biofilm* (2000) Environ Microbiol 2(6) DOI: 10.1046/j.1462-2920.2000.00150.x

SeQuant, A Practical Guide to Ion Chromatography - An Introduction and Troubleshooting Manual, p. 3–5, 2007. DOI:10.1002/978352761324

Ytrestøyl, T., Takle, H., Kolarevic, J., Calabrese, S., Rosseland, B. O., Teien, H. -C., Nilsen, T. O., Stefansson, S., Handeland, S. O. & Terjesen, B. F. (2014) *Effekt av saltholdighet og trening på vekst, fysiologi og velferd hos sto postsmolt av Atlantisk laks i RAS.* In Abstracts RCN Program conference Mariculture 2014, p. 59. Tromsø, Norway, March 31st - April 2nd 2014

Weiss,W. *Review of Microbially Induced Corrosion and Comments on Needs Related to Testing Procedures* 2014 Conference paper DOI: 10.5703/1288284315388

Worden, R. . *Analytical Methods* 2005 Encyclopedia of Geology (54-74) Elesiver https://doi.org/10.1016/B0-12-369396-9/00096-4

Appendix

A.1 All measurements of pH, salinity and conductivity

Table A.1.1 Water quality parameters pH, salinity and conductivity measurements for each sample point and date.

Date	Sample point	pH	Salinity %	Cond. [mS/sm]	Date	Sample point	pH	Salinity %	Cond. [mS/sm]
18.03.2020	RAS1(A)	7,809	11,9	20,3	18.03.2020	RAS1 sump	7,857	11,9	20,3
25.03.2020		7,802	11,3	19,25	25.03.2020		7,925	11,3	19,28
01.04.2020		7,792	12	20,3	01.04.2020		7,892	12	20,4
07.04.2020		7,66	12,4	21,1 µS/cm	07.04.2020		7,83	12,4	21,1
15.04.2020		7,581	12,7	21,5	15.04.2020		7,84	12,8	21,6
22.04.2020		7,72	12,1	20,6	22.04.2020		7,849	12,1	
29.04.2020		7,643	12,5	21,1	29.04.2020		7,851	12,4	21,1
07.05.2020		7,76	12,6	21,4	07.05.2020		7,89	12,6	21,3
13.05.2020		7,605	12,3	21	13.05.2020		7,843	12,3	21
20.05.2020		7,606	12	20,5	20.05.2020		7,942	12,1	20,5
27.05.2020		7,628	12,1	20,6	27.05.2020		7,935	12,1	20,6
03.06.2020		7,515	12	20,4	03.06.2020		7,849	12	20,4
10.06.2020		7,618	12,1	20,5	10.06.2020		7,855	12,1	20,5
18.03.2020	RAS1(B)	7,768	11,4	19,55	18.03.2020	RAS2 sump	7,775	11,4	19,56
25.03.2020		7,68	11,9	20,3	25.03.2020		7,803	11,9	20,3
01.04.2020		7,589	11,8	20	01.04.2020		7,71	11,8	20
07.04.2020		7,68	11,5	19,55	07.04.2020		7,812	11,5	19,56
15.04.2020		7,64	12,5	21,1	15.04.2020		7,779	12,4	21,1
22.04.2020		7,632	11,4	19,4	22.04.2020		7,775	11,4	
29.04.2020		7,501	11,9	20,2	29.04.2020		7,7	11,9	20,2
07.05.2020		7,52	12,4	21,1	07.05.2020		7,71	12,4	21,1
13.05.2020		7,504	12	20,4	13.05.2020		7,749	12	20,4
20.05.2020		7,497	11,9	20,3	20.05.2020		7,841	11,9	20,3
27.05.2020		7,555	12,5	21,2	27.05.2020		7,876	12,5	21,2
03.06.2020		7,373	11,9	20,2	03.06.2020		7,68	11,9	20,2
10.06.2020		7,487	11,4	19,45	10.06.2020		7,712	11,4	19,4
18.03.2020	RAS2(C)	7,868	11,9	20,3	18.03.2020	FW	7,088	0,1	420 µS/cm
25.03.2020		7,815	11,3	19,27	25.03.2020		7,142	0,1	**438 µS/cm**
01.04.2020		7,802	12	20,3	01.04.2020		7,077	0,1	476 µS/cm
07.04.2020		7,718	12,4	21,1	07.04.2020		7,344	0	290 µS/cm
15.04.2020		7,718	12,7	21,5	15.04.2020		7,437	0,1	333 µS/cm
22.04.2020		7,713	12,1	20,6	22.04.2020		7,274	0	291 µS/cm
29.04.2020		7,698	12,5	21,1	29.04.2020		7,082	0,1	451 µS/cm
07.05.2020		7,74	12,6	21,3	07.05.2020		7,3	0,1	333 µS/cm
13.05.2020		7,627	12,3	21	13.05.2020		7,257	0,1	339 µS/cm
20.05.2020		7,645	12	20,5	20.05.2020		7,317	0,1	330 µS/cm
27.05.2020		7,63	12,1	20,6	27.05.2020		7,301	0	296 µS/cm
03.06.2020		7,521	12	20,4	03.06.2020		7,25	0	294 µS/cm
10.06.2020		7,62	12,1	20,5	10.06.2020		7,94	0	238µS/cm
18.03.2020	RAS2(D)	7,798	11,4	19,55	18.03.2020	Nf,in	7,984	33,6	52,1
25.03.2020		7,681	11,9	20,2	25.03.2020		7,973	33,7	52,1
01.04.2020		7,594	11,8	20	01.04.2020		7,959	33,6	52,1
07.04.2020		7,706	11,5	19,55	07.04.2020		7,97	33,2	51,6
15.04.2020		7,692	12,4	21,1	15.04.2020		8,046	12,9	21,8
22.04.2020		7,654	11,4	19,45	22.04.2020		7,927	34	
29.04.2020		7,484	11,9	20,3	29.04.2020		7,934	34	52,5
07.05.2020		7,54	12,4	21,1	07.05.2020		7,91	34,2	53
13.05.2020		7,514	12	20,4	13.05.2020		7,916	34,1	52,8
20.05.2020		7,513	12	20,4	20.05.2020		7,938	33,7	52,6
27.05.2020		7,565	12,5	21,2	27.05.2020		7,959	33,9	52,5
03.06.2020		7,275	11,9	20,2	03.06.2020		7,894	33,8	52,3
10.06.2020		7,476	11,4	19,45	10.06.2020		7,872	34	52,2
18.03.2020	Nf,out(2)	8,058	11,3	13,33	18.03.2020	Nf,out(1)	8,107	11,4	19,61
25.03.2020		8,101	11,9	20,3	25.03.2020		8,103	12,3	20,9
01.04.2020		8,098	12	20,5	01.04.2020		8,091	12,4	21,1
07.04.2020		8,121	12	20,5	07.04.2020		8,131	12,4	21,1
15.04.2020		8,039	12,2	20,8	15.04.2020		7,918	33,8	52,2
22.04.2020		8,054	11,9		22.04.2020		8,068	12,7	
29.04.2020		8,011	11,7	20	29.04.2020		8,091	12,9	21,8
07.05.2020		8,04	12,5	21,2	07.05.2020		8,03	12,9	21,8
13.05.2020		8,035	10,3	17,87	13.05.2020		8,064	11,6	19,95
20.05.2020		8,043	11,9	20,3	20.05.2020		8,098	12,5	21,3
27.05.2020		8,088	10,8	18,62	27.05.2020		8,119	11,7	19,92
03.06.2020		8,01	11,6	19,83	03.06.2020		8,024	12,4	21
10.06.2020		7,973	11,9	20,2	10.06.2020		7,954	12,9	21,8

A.2 Measurements done by ICP-MS for magnesium, silicone, sulfur, potassium, calsium, bromide and strontium

Table A.2.1 Measured concentration for element and each sample point by ICP-MS analysis.

Sulphur — Concentration [microgram/L] per Sample point

Date	FW	NF,in	NF,out(1)	NF,out(2)	RAS1,Sump	RAS1,Tank(A)	RAS1,Tank(B)	RAS2,Sump	RAS2,Tank(C)	RAS2,Tank(D)
11.03.20	7991	823500	22089	19433	20288	16860	21026	267735	268481	269293
18.03.20	9416	852023	23473	-	23004	22120	22694	262313	252342	255816
25.03.20	9685	829137	24756	23235	23703	23700	22973	282479	280073	277173
01.04.20	9595	820907	22058	21754	24340	23400	23520	292692	276926	274896
07.04.20	9324	817205	22954	23101	24368	25037	25336	275564	276474	268699
15.04.20	9837	884867	24370	22503	24436	24973	25264	290995	301897	298514
22.04.20	9377	876950	24302	23277	23578	24659	24552	254615	267554	264417
29.04.20	16023	1314804	36679	34684	37946	36890	39824	428411	439163	448858
07.05.20	14169	1234152	32805	31617	34167	36480	38973	415918	460537	437472
13.05.20	9236	1047661	26519	23078	26858	37169	31842	310236	409007	373734
20.05.20	9352	919066	25873	23763	26488	36519	26894	299025	316670	309199
27.05.20	7996	827569	24158	22579	23440	26298	26585	282086	317169	299605
03.06.20	9360	834448	25516	23764	23991	27419	28513	264770	270145	298270
10.06.20	6104	780594	23007	23143	22870	24476	24621	-	253484	255728

Silicone — Concentration [microgram/L] per Sample point

Date	FW	NF,in	NF,out(1)	NF,out(2)	RAS1,Sump	RAS1,Tank(A)	RAS1,Tank(B)	RAS2,Sump	RAS2,Tank(C)	RAS2,Tank(D)
11.03.20	2158	-	113	17	12	68	84	2401	2471	2482
18.03.20	5128	-	129	76	139	100	100	3877	2805	2796
25.03.20	5408	-	98	65	143	117	139	4017	3905	3926
01.04.20	5799	-	90	73	154	146	141	4214	4051	4013
07.04.20	7543	-	83	66	160	153	162	4596	4778	4853
15.04.20	7577	-	75	169	140	133	139	4403	4665	4470
22.04.20	7825	-	156	62	62	69	62	4295	4787	4634
29.04.20	8764	-	289	123	98	282	98	7156	7242	6902
07.05.20	10749	-	310	135	163	172	182	6684	7264	6424
13.05.20	8568	-	235	78	194	277	235	5412	7177	5780
20.05.20	8331	-	165	63	224	262	258	4969	5958	4081
27.05.20	6084	-	116	44	198	241	467	3814	4347	4357
03.06.20	7680	-	144	56	274	263	288	3929	3888	3993
10.06.20	5287	-	189	320	75	340	323	295	3987	-

Calcium — Concentration [microgram/L] per Sample point

Date	FW	NF,in	NF,out(1)	NF,out(2)	RAS1,Sump	RAS1,Tank(A)	RAS1,Tank(B)	RAS2,Sump	RAS2,Tank(C)	RAS2,Tank(D)
11.03.20	7131	403290	74455	64337	70060	57837	73251	115492	118750	118050
18.03.20	10613	435676	97934	-	97715	79488	78980	146708	111964	113070
25.03.20	10592	406505	105012	103302	91360	90462	91196	159853	155555	155862
01.04.20	10730	398514	120314	121791	106742	102699	104029	160444	156515	154896
07.04.20	12142	399253	120195	112147	111912	110250	114257	150818	152693	155003
15.04.20	12099	122070	480790	117625	109015	110903	113637	153745	160063	155021
22.04.20	12596	434484	105108	97136	92739	104468	98365	126721	140111	137490
29.04.20	11823	496911	118648	106289	112514	108973	112726	158129	160283	-
07.05.20	10895	488342	118187	113260	106970	111068	115684	162801	174952	169829
13.05.20	9829	447150	96158	79973	97306	108258	103249	143355	166724	154861
20.05.20	9926	426692	92060	96865	85214	96298	93915	128571	150587	146178
27.05.20	7932	376299	79137	72585	79625	83086	84126	127767	140675	131227
03.06.20	9701	384327	88910	81624	77796	78765	80017	120778	121896	125232
10.06.20	6897	360202	72670	80201	87639	76278	77040	73881	113230	114580

Strontium — Concentration [microgram/L] per Sample point

Date	FW	NF,in	NF,out(1)	NF,out(2)	RAS1,Sump	RAS1,Tank(A)	RAS1,Tank(B)	RAS2,Sump	RAS2,Tank(C)	RAS2,Tank(D)
11.03.20	65	7401	1503	1362	1573	1209	1526	2474	2513	2496
18.03.20	68	7179	1446	-	1469	1473	1484	2311	2264	2308
25.03.20	69	7014	1570	1499	1368	1374	1357	2435	2365	2439
01.04.20	73	7055	1683	1622	1539	1462	1557	2439	2372	2403
07.04.20	63	7228	1673	1608	1610	1575	1615	2311	2380	2390
15.04.20	64	1750	7421	1694	1649	1664	1659	2591	2564	2520
22.04.20	64	7104	1818	1575	1557	1575	1528	2332	2288	2283
29.04.20	88	8804	2079	1918	2025	1929	2035	2815	2830	-
07.05.20	73	9084	2168	2090	1941	2007	2095	2996	3161	3047
13.05.20	68	8480	1865	1531	1851	1966	1893	2730	3033	2871
20.05.20	69	8205	1867	1893	1729	1856	1809	2617	2882	2782
27.05.20	57	7779	1717	1565	1715	1737	1764	2799	2909	2798
03.06.20	67	8098	1890	1714	1627	1724	1660	2681	2690	2625
10.06.20	48	8063	1684	1757	1932	1690	1677	1654	2410	2425

Magnesium — Concentration [microgram/L] per Sample point

Date	FW	NF,in	NF,out(1)	NF,out(2)	RAS1,Sump	RAS1,Tank(A)	RAS1,Tank(B)	RAS2,Sump	RAS2,Tank(C)	RAS2,Tank(D)
11.03.20	4653	1073623	230099	231861	231861	177848	227340	377582	376030	375339
18.03.20	5791	1127046	239973	239973	238385	224731	227834	366983	345342	346796
25.03.20	-	-	258414	217527	217527	224088	220724	384276	380918	379193
01.04.20	7009	1085340	271901	244127	244127	242125	245141	390048	382341	378661
07.04.20	3441	1070985	270985	257799	257799	259064	268082	362100	373925	365061
15.04.20	3543	1180276	279478	259917	259917	270861	273469	393068	397109	394259
22.04.20	3364	1146470	261716	242987	242987	250414	243958	340779	346882	355430
29.04.20	7908	1571700	379493	376355	376355	368351	376445	501468	526030	-
07.05.20	4210	1489143	365278	332831	332831	352076	374742	489679	554549	530521
13.05.20	3381	1336768	285590	289136	289136	364080	306826	421215	484469	442955
20.05.20	3518	1233129	266401	255304	255304	328511	277248	384993	435470	424466
27.05.20	2785	1024922	226379	229186	229186	285483	251079	366505	407734	391678
03.06.20	3559	1114172	262643	228842	228842	255364	250805	362924	353609	387860
10.06.20	-	1007004	222025	242484	242484	233532	233564	213369	325830	334047

Potassium — Concentration [microgram/L] per Sample point

Date	FW	NF,in	NF,out(1)	NF,out(2)	RAS1,Sump	RAS1,Tank(A)	RAS1,Tank(B)	RAS2,Sump	RAS2,Tank(C)	RAS2,Tank(D)
11.03.20	2365	359926	120346	114090	123681	93870	120702	116773	116675	117622
18.03.20	2893	350560	120732	-	-	120112	120815	112133	108517	109045
25.03.20	3050	345564	128444	124686	119229	120555	118565	119562	118364	119049
01.04.20	3132	344207	127970	127473	129264	127759	128873	123380	120022	119698
07.04.20	2623	341451	126882	124008	133448	131651	138967	116753	118575	117464
15.04.20	2691	130439	356887	125060	132825	138119	137558	127742	128838	127605
22.04.20	2653	358522	124664	123524	128417	130299	128289	113057	114402	117976
29.04.20	-	-	-	-	-	-	-	-	-	-
07.05.20	-	-	-	-	-	-	-	-	-	-
13.05.20	-	-	-	-	-	-	-	-	-	-
20.05.20	-	-	-	-	-	-	-	-	-	-
27.05.20	-	-	-	-	-	-	-	-	-	-
03.06.20	-	-	-	-	-	-	-	-	-	-
10.06.20	-	-	-	-	-	-	-	-	-	-

Bromide — Concentration [microgram/L] per Sample point

Date	FW	NF,in	NF,out(1)	NF,out(2)	RAS1,Sump	RAS1,Tank(A)	RAS1,Tank(B)	RAS2,Sump	RAS2,Tank(C)	RAS2,Tank(D)
11.03.20	274	71852	25821	24565	26933	19700	25304	22930	23158	23483
18.03.20	280	70549	25413	-	26162	25758	26395	22302	21823	22087
25.03.20	309	70456	27407	26824	25481	25287	25272	23450	23061	23162
01.04.20	328	69650	27340	26551	26833	26535	26623	23805	22861	23046
07.04.20	194	70560	27065	26632	27421	27275	28374	22253	22629	22224
15.04.20	187	28072	71533	26575	27720	28315	28446	24556	24184	24134
22.04.20	180	72593	27389	26274	26359	26736	26046	21615	21555	22006
29.04.20	809	81207	30970	29063	31762	30322	30842	25818	26634	-
07.05.20	816	80943	31308	30190	30730	31948	32921	27319	28185	26943
13.05.20	352	77892	25484	21624	28175	32805	29722	23904	27538	26368
20.05.20	819	75331	28130	26800	27165	27593	28443	23592	24923	24642
27.05.20	746	68946	25845	23564	26076	27950	27781	24478	25530	24772
03.06.20	698	67294	27912	25605	25975	26922	26194	22475	23647	23553
10.06.20	694	67932	26557	25443	27052	26165	25449	25684	21880	21474

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A.3 All measured values of sulfate measured by IC-Analysis

Table A.3 Measured sulfate concentration (mg/L) for each fish tank measured by IC-analysis

Date	Concentration, mg/L											
	RAS1,Tank(A)			RAS2,Tank(C)			RAS1,Tank(B)			RAS2,Tank(D)		
25.mar	12	45,16		90,283	459,15		1357,358			1242,831	734,2	
01.apr	105,605	8,17		1259,633			98,882	15,23		1277,554	302,5	
07.apr	5,372	69,48		11,694	593,1		264,246	74,79			901,8	
15.apr	102,97	13,61		1381,601	1399,65		105,455	82,08		1672,786	1369,55	
22.apr		105,65	782,2	10,648	700,51	1663,6	142,075	78,01	766,88		754,31	966,65
29.apr	96,048	159,18		902,894			117,274	68,06		1393,893	1033,85	
07.mai	4,377	63,1			226,8			21,42		0,435	767,35	
13.mai	114,504	89,66		1335,032	1069,45		106,449	69,47		1212,715	538,35	
20.mai		138,43		60,393	827		5,898	12,9		86,365	896,65	
27.mai	106,371	44,5		1485,36	1152,5		107,667	31,6		1389,383	1269,3	
03.jun	32,407	14,6		8,66	7228,7		1,946	93,48		189,187	1,6	
10.jun	126,231									13,487	1539,25	

A.4 All measurements of absorbance at 254 nm for each sampling point and date by UV-Vis

Table A.4 Absorbance measured for each sampling point and date by UV-Vis analysis

Dato	Absorbance at 254 nm									
	NF,in	NF,out(1)	NF,out(2)	FW	RAS1,Sump	RAS2,Sump	RAS1,Tank(A)	RAS2,Tank(C)	RAS1,Tank(B)	RAS2,Tank(D)
18.mar	0,016	0,005	0,005	0,015	0,011	0,020	0,015	0,019	0,017	0,024
25.mar	0,017	0,010	0,011	0,017	0,018	0,017	0,020	0,021	0,022	0,026
01.apr	0,018	0,010	0,012	0,020	0,026	0,026	0,026	0,029	0,035	0,027
07.apr	0,030	0,015	0,016	0,021	0,071	0,021	0,025	0,037	0,036	0,033
15.apr	0,017	0,024	0,017	-	0,037	0,039	0,044	0,047	0,059	0,044
22.apr	0,025	0,017	0,018	0,026	0,050	0,044	0,042	0,045	0,043	0,046
29.apr	0,026	0,021	0,020	0,028	0,051	0,048	0,053	0,056	0,037	0,045
07.mai	0,024	0,017	0,017	0,026	0,050	0,041	0,047	0,050	0,048	0,057
13.mai	0,023	0,017	0,019	0,024	0,040	0,050	0,050	0,049	0,047	0,045
20.mai	0,028	0,018	0,018	0,026	0,070	0,076	0,063	0,089	0,069	0,085
27.mai	0,022	0,022	0,021	0,029	0,079	0,061	0,084	0,086	0,059	0,063
03.jun	0,030	0,020	0,019	0,029	0,069	0,074	0,082	0,071	0,067	0,069
10.jun	0,030	0,021	0,022	0,031	0,087	0,109	0,087	0,095	0,101	0,081