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The Effects of Membrane Filtration in a Recirculating Aquaculture System on Water Quality and Fish Performance of Atlantic Salmon (*Salmo salar*)

Gaute Alexander Nedberg Helberg

Marine Coastal Development
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Supervisor: Rolf Erik Olsen, IBI
Co-supervisor: Trond Rosten, SINTEF

Norwegian University of Science and Technology
Department of Biology

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Abstract

Recirculating aquaculture systems (RAS) could be a solution to several of the challenges associated with environmentally sustainable growth of the aquaculture industry. However, high investment and operating costs are associated with RAS, and intensification through reduction of water usage and increasing biomass densities might be necessary to increase economic feasibility. Furthermore, intensification is linked with risks of accumulation of compounds such as small particles not removed by traditional water treatment, which reduce the quality of the water, and may affect the performance and quality of the fish. There is currently limited knowledge on how particles affect fish performance in RAS, and how different removal efficiencies of small particles may affect water quality development. Membrane filtration could be used to remove small particles and bacteria, although the effects on production of Atlantic salmon (*Salmo salar*) is not thoroughly studied.

The aim of this thesis was to investigate how utilisation of a membrane filter for particle removal in RAS affects water quality development and fish performance of Atlantic salmon. Two pilot-scale RAS were compared; one system using conventional water treatment components (cRAS), and one including a membrane filtering 10% of the circulated water (mRAS). Water quality parameters (temperature, oxygen, salinity, carbon dioxide, pH, alkalinity, total ammonia nitrogen, nitrite, nitrate, turbidity and total suspended solids) and fish performance parameters (weight, length, morphological welfare indicators, blood values, smoltification indicators and recovery from handling stress) were measured at selected time points over the course of 18 weeks, and results from cRAS and mRAS were compared. Unforeseen circumstances forced changes in operational conditions during the experiment, dividing the study into distinct periods; two with low particulate load, and two with high particulate load. The observed differences in water quality caused by membrane filtration, was primarily lower turbidity due to increased removal of particles, and increased water temperature due to the production of heat caused by operating the membrane. Dissolved oxygen and carbon dioxide levels were also different, which could be coupled to the diverging biomasses in the systems. Higher growth occurred in mRAS, likely the result of a higher water temperature. The biggest discrepancy in fish performance was growth, making it difficult to conclude whether membrane filtration would be beneficial from a fish performance perspective had the temperature been controlled. If the effect on temperature is taken into account and utilised, membrane filtration could prove to be a good addition in a RAS for particle removal purposes.

Sammendrag

Resirkulerende akvakultursystemer (RAS) kan være en løsning på flere av utfordringene tilknyttet bærekraftig vekst av akvakulturnæringen. RAS har store investerings- og driftskostnader, og intensivering av produksjonen, ved å redusere vannforbruk og øke biomassetettheten, er muligens nødvendig for å gjøre det økonomisk gunstig. En slik intensivering øker risikoen for akkumulering av stoffer, slik som små partikler som ikke fjernes gjennom tradisjonell vannbehandling, som vil redusere vannkvaliteten og kan påvirke fisken. Det er for øyeblikket begrenset kunnskap om hvordan partikler påvirker fisk i RAS, og hvordan ulik grad av partikkelfjerning påvirker utviklingen av vannkvalitet. Membranfiltrering kan brukes til å fjerne små partikler og bakterier, men effekten det har på produksjon av atlantisk laks (*Salmo salar*) er ikke nøye studert.

Målet med denne masteroppgaven var å undersøke hvordan bruk av membranfiltrering for partikkelfjerning i RAS påvirker utvikling av vannkvalitet og fiskeytelse hos atlantisk laks. To små-skala RAS ble sammenlignet: ett system med konvensjonelle vannbehandlingskomponenter (cRAS) og ett som inkluderte en membran som filtrerte 10% av vannstrømmen (mRAS). Vannkvalitetsparametere (temperatur, oksygen, salinitet, karbondioksid, pH, alkalinitet, total ammonium nitrogen, nitritt, nitrat, turbiditet og totalt suspendert tørrstoff) og fiskeytelsesparametere (vekt, lengde, morfologiske velferdsindikatorer, blodverdier, smoltifiseringsindikatorer og evne til å komme seg etter håndteringsstress) ble målt på selekterte tidspunkt over 18 uker, og resultatene fra cRAS og mRAS ble sammenlignet. Uforutsette hendelser førte til at forsøket ble delt i perioder med ulik drift; to perioder med høy partikkelbelastning og to perioder med lav partikkelbelastning. De observerte effektene av membranfiltrering på vannkvalitet, var primært lavere turbiditet som et resultat av økt partikkelfjerning, og høyere temperatur som en konsekvens av varmeproduksjon ved drift av membranfilteret. Oksygen- og karbondioksid-nivåer var også forskjellige, sannsynligvis koblet til økende forskjeller i biomasse i systemene. Det var høyere vekst i mRAS, hovedsakelig på grunn av den høyere vanntemperaturen. Den største forskjellen i fiskeytelse var vekst, og det er derfor vanskelig å konkludere om membranfiltrering ville vært gunstig for fiskeytelse om temperaturen hadde vært kontrollert. Dersom effekten på temperatur tas hensyn til, kan membranfiltrering for partikkelfjerning fungere godt i RAS.

Table of contents

List of figures IX

List of tables X

Abbreviations XI

1 Introduction 1
1.1 Developmental trends in aquaculture 1
1.1.1 The role of recirculating aquaculture systems 1
1.2 The importance of water quality in RAS 2
1.2.1 Temperature, oxygen and salinity 3
1.2.2 Nitrogenous compounds 4
1.2.3 Carbon dioxide, pH and alkalinity 5
1.2.4 Particles 5
1.3 Membrane filtration 6
1.4 Experimental aims 7

2 Materials and methods 9
2.1 Water treatment 9
2.2 Rearing conditions 11
2.3 Fish sampling and analyses 12
2.3.1 Sedation, euthanizing and blood sampling 12
2.3.2 Recovery from handling stress test 12
2.3.3 Seawater tolerance test 13
2.3.4 Blood sample analysis 13
2.3.5 Morphology 13
2.3.5.1 External examination 13
2.3.5.2 Smoltification 14
2.3.5.3 Internal examination 14
2.3.6 Growth 14
2.4 Water quality sampling and analyses 15
2.4.1 Turbidity 15
2.4.2 Total suspended solids 16
2.4.1 Temperature, oxygen and salinity 17
2.4.1 Total ammonia nitrogen, nitrite and nitrate 17
2.4.2 Alkalinity, pH and CO_2 17
2.5 Statistics 17

3 Methodological considerations 19

4 Results 21
4.1 Water quality development 21
4.1.1 Recirculation, feed, turbidity and TSS 21
4.1.2 Temperature, oxygen and salinity 23
4.1.3 TAN, nitrite and nitrate 25
4.1.4 CO_2, pH and alkalinity 26
4.1.5 Water quality summary 27
4.2 Fish performance development 27
4.2.1 Weight, length, condition factor and growth 27
4.2.2 Morphology 29
4.2.3 Recovery from handling- and seawater tolerance tests 32

5 Discussion 35
5.1 Turbidity, and associated effects 35
5.1.1 Effects on other water quality parameters 36
5.1.2 Effects on development of biofilter efficiency 37
5.1.3 Effects on fish performance 38
5.2 Temperature development, and associated effects 40
5.2.1 Effects on growth 40
5.2.2 Smoltification 41
5.3 Oxygen, carbon dioxide, and associated effects 42
5.3.1 Oxygen 42
5.3.2 Carbon dioxide 43
5.4 Other developmental trends worth noting 45
5.4.1 Response to handling stress 45
5.4.2 Mortality 45
5.4.3 Other examined morphological welfare indicators 45
5.4.4 Salinity 47
5.4.5 Other potential stressors 47
6 Conclusions and further recommendations 49
7 References 51
8 Appendix I 57
9 Appendix II 58

List of figures

Fig. 1 Schematic overview of the experimental setup……..…10
Fig. 2 Periods within the experiment with different operational conditions…….20
Fig. 3 Development of recirculation, added feed and turbidity……....22
Fig. 4 Development of temperature, oxygen and salinity…….24
Fig. 5 Development of total ammonia nitrogen, nitrite and nitrate……...25
Fig. 6 Development of carbon dioxide, pH and total alkalinity……26
Fig. 7 Development of weight, length and condition factor…….28
Fig. 8 Development of morphological welfare scores……..30
Fig. 9 Plasma glucose and lactate concentrations from handling stress tests……33

List of tables

Table 1 Average starting weight and mean biomass density per system at stocking……11
Table 2 Water quality in recovery tanks……...13
Table 3 Scoring index for evaluation of morphological welfare parameters……14
Table 4 TSS measurements from S1……23
Table 5 Summary of water quality parameters (mean±SD)……27
Table 6 Growth rates over the entire study period……29
Table 7 Growth rates over the final period with high particulate load……29
Table 8 Summary of morphological welfare parameters……31
Table 9 Summary of severity of observed damages……32
Table 10 Plasma chloride, seawater tolerance test……32
Table 11 Overview of initial weight, density and number of fish in each tank……...Appendix I
Table 12 Overview of all fish samplings……...…….Appendix II

Abbreviations

A	Acclimatisation period prior to start of experiment
BF	Biofilter
cRAS	Conventional recirculating aquaculture system
DOC	Dissolved organic carbon
DS	Drum screen filter
FCR	Feed conversion ratio
FT	Fish tank
MF	Membrane filter
mRAS	Membrane-equipped recirculating aquaculture system
NTNU	Norwegian University of Science and Technology
NTU	Nephelometric turbidity units
P1	Period with low particular load (week 0-5 of the experiment)
P2	Period with high particular load (week 5-10 of the experiment)
P3	Period with low particular load (week 11-14 of the experiment)
P4	Period with high particular load (week 15-18 of the experiment)
POM	Particulate organic matter
RAS	Recirculating aquaculture system
SD	Standard deviation
SGR	Specific growth rate
TAN	Total ammonia nitrogen
TGC	Thermal growth coefficient
TOC	Total organic carbon
TSS	Total suspended solids
UV	Ultraviolet

1 Introduction

1.1 Developmental trends in aquaculture

Aquaculture is an industry in growth, with the potential to be environmentally sustainable (FAO, 2016). As aquaculture production expands, certain aspects require more focus if already existing and potentially upcoming problems are to be solved and sustainability ensured (Diana et al., 2013). The magnitude of current and future issues is in part dependent on the location of cultivation, as well as the species being produced. On a world basis, these problems may include topics such as fresh water usage, waste management and diseases (Diana et al., 2013). In Norway, a recent risk assessment report covering the environmental impacts of aquaculture and fish welfare, emphasised issues the authorities need advice about (Grefsrud et al., 2018). The report listed several important topics, including escapes and genetic interaction, emissions of nutrient and organic waste and salmon lice. A technology that could play a central role in overcoming the problems, and enabling sustainable growth of aquaculture, is recirculating aquaculture systems (RAS) (Martins et al., 2010, Dalsgaard et al., 2013, d'Orbcastel et al., 2009a).

1.1.1 The role of recirculating aquaculture systems

Recirculating aquaculture systems (RAS) can be defined as systems where the outlet water from fish tanks is treated and re-used instead of being released into a recipient water body (Lekang, 2013). Based on the degree of re-use of water, different water treatment technologies may be appropriate to maintain water quality parameters within acceptable levels. Addition of oxygen, removal of carbon dioxide by degassing, adjustments of pH and alkalinity by adding buffers, conversion of nitrogenous wastes with biofilters and removal of solids with mechanical filters are all common practices (Lekang, 2013).

The utilisation of RAS enables a potential for more constant water quality compared to traditional flow-through systems without re-use of water, which in turn can have a positive effect on growth and welfare (d'Orbcastel et al., 2009b). It can also reduce water consumption of freshwater aquaculture down to sustainable levels (Verdegem et al., 2006). Under certain conditions, RAS for production of Atlantic salmon (*Salmo salar*) can reduce the total negative environmental impacts compared to production in traditional open net pen systems (Liu et al., 2016). RAS-technology also makes entirely land-based farming of Atlantic salmon more

feasible, enabling production in new areas with the potential of locally grown fish of market competitive quality (Badiola et al., 2017). One of the potentially biggest challenges to sustainable RAS is the high capital costs, and large scale intensive productions are suggested to reduce the investment and operation costs (Dalsgaard et al., 2013). However, intensification and reduction of water usage may increase the risk of accumulation of potentially harmful substances (Davidson et al., 2009, Martins et al., 2009, Martins et al., 2010), including hormones (Mota et al., 2014, Mota et al., 2017a, Mota et al., 2017b) and small particles (Davidson et al., 2009, Chen et al., 1993, Patterson and Watts, 2003).

Particles with a low density do not settle, but stay as suspended solids in the water (Chiam and Sarbatly, 2011). A common practice for mechanical removal of suspended solids in RAS is the use of rotating microscreen filters, where a screen mesh pore size of 40 or 60µm is often used (Cripps and Bergheim, 2000). In high-intensive reuse systems with little water exchange, the result is that fine particles (< 20µm) accumulate (Davidson et al., 2009, Patterson and Watts, 2003). Results from Chen et al. (1993) indicate that particles with a diameter less than 20 µm can constitute more than 95% of the suspended solids in RAS. Colt (2006) stated that the potential impact of small particles and organic compounds is the point of greatest uncertainty for water quality in high-intensive RAS.

1.2 The importance of water quality in RAS

Fish are particularly sensitive to the water they live in, mainly due to their delicate gills being exposed to any chemicals, aquatic pollutants or external factors present in the water which might cause stress (Bonga, 1997). Consequently, they require a certain quality of the water surrounding them. The limits for adequate water quality in RAS can vary between species, and for different life stages within the same species (Colt, 2006). This is the case for Atlantic salmon, which has a juvenile freshwater stage (parr) which go through several developmental changes (smoltification) that enables them to osmoregulate in seawater, where they develop into an adult which when mature will return to fresh water to spawn (Wedemeyer, 1996). The different life stages require different water quality, and the water quality may also affect life stage development (Wedemeyer, 1996).

The nitrification efficiency (conversion of toxic ammonia and nitrite) of the biofilter also depends on water quality. The optimum water quality for salmon and nitrifying bacteria do not

correspond, meaning that certain trade-offs must be taken for some variables. In addition, for industrial aquaculture purposes, there is an economic perspective to water quality. The cost of building and operating a RAS that can maintain a certain water quality compared to the economic gain of rearing the fish in optimum water, will determine how feasible it is that the water treatment technology responsible for the water quality will be applied. Although water quality management can be costly, some parameters are paramount to maintain within acceptable ranges to ensure growth and fish welfare.

1.2.1 Temperature, oxygen and salinity

Temperature has several direct effects on Atlantic salmon, affecting growth (Austreng et al., 1987) and playing different roles in life stage development. By affecting the rate of development and by interactions with the photoperiod, temperature plays a role in the timing of smolting (McCormick et al., 2002), and influences timing of seaward smolt migration (Jonsson and Ruudhansen, 1985). Increased water temperature is also argued to be related to early maturation of Atlantic salmon (Good and Davidson, 2016).

In addition, temperature interacts with several other water quality parameters. Increased water temperature reduces the available dissolved oxygen by affecting the solubility, it increases oxygen consumption and metabolic rates, and it can increase the toxicity of dissolved contaminants (Wedemeyer, 1996). The combination makes temperature one of the most important environmental variables to control. Atlantic salmon can tolerate a wide range of temperatures, but the optimum temperature will vary with life stage (Noble et al., 2018) and avoiding rapid water temperature changes is of paramount importance (Wedemeyer, 1996).

Another important environmental variable is dissolved oxygen. In aquaculture, the main problem has traditionally been to maintain high enough saturation, thus welfare recommendations focus on the lower limits of dissolved oxygen (Noble et al., 2018). Although it is rarely regarded as an issue in intensive aquaculture, it is important to be aware that too high concentrations can also cause severe health impediments (Espmark and Baeverfjord, 2009, Espmark et al., 2010). The solubility of oxygen in water is affected by other factors in addition to the previously mentioned temperature, such as salinity. Salinity preferences for Atlantic salmon varies with life stage. In RAS, some salinity is recommended even for the early freshwater stages, because chloride ions protect against the toxicity of nitrite (Noble et al.,

2018). Salinity also affects bacterial composition, and rapid changes in salinity can reduce the nitrification rate of the biofilters (Colt, 2006).

1.2.2 Nitrogenous compounds

With the high biomass densities that is common in aquaculture, there is an increased probability that the fish will be exposed to the potentially degenerative nitrogenous wastes they excrete (Tomasso, 1994). To reduce and remove these wastes, recirculation systems utilise biofilters with nitrifying bacteria that oxidize ammonium to nitrite (NO_2^-) and nitrate (NO_3^-) (Lekang, 2013). There are several biofilter designs, such as fixed bed and moving bed biofilters, primarily aimed at increasing the available surface area that nitrifying bacteria can attach to.

Ammonia is released through bacterial decomposition of organic matter, and is also excreted by the fish. The total ammonia nitrogen (TAN) in the water consists of both a unionized (NH_3) and an ionized (NH_4^+) form, with the unionized being the most toxic. The most important factor determining the ratio of the unionized/ionized forms is pH, with high pH increasing the presence of the unionized form and thus the toxicity of TAN (Wedemeyer, 1996). Recommended maximum levels of TAN therefore depend on pH, but also on other water quality parameters such as temperature, salinity, hardness and alkalinity (Thorarensen and Farrell, 2011).

Ammonia is converted to nitrite (NO_2^-) by ammonia-oxidising bacteria in the biofilter. Nitrite is also toxic, and as with ammonia, there as several variables that interact in determining the toxicity of nitrite (Kroupova et al., 2005). Nitrite reduces the oxygen-carrying capacity of the blood, and competes with chloride for uptake through chloride cells of the gills (Lewis and Morris, 1986, Kroupova et al., 2005, Tomasso, 1994). This means that toxicity of nitrite is reduced with increased salinity.

Nitrite is further converted to nitrate (NO_3^-) by nitrite-oxidising bacteria in the biofilter. Nitrate has in many cases been considered nontoxic to fish (Wedemeyer, 1996), but in intensive RAS it might be necessary to remove nitrate to avoid severe accumulation. Denitrification for removal of nitrate is possible, with bacteria converting nitrate into elementary nitrogen (N_2), but requires an anoxic environment and addition of organic matter (Van Rijn et al., 2006).

1.2.3 Carbon dioxide, pH and alkalinity

Carbon dioxide (CO_2) is continuously produced by the fish and during microbial decomposition of organic matter (Wedemeyer, 1996). The amount of CO_2 in the water depends on temperature, with higher temperature reducing the solubility of CO_2. Toxicity of CO_2 is reduced in alkaline water, due to conversion of dissolved CO_2 to nontoxic bicarbonate and carbonate ions (Wedemeyer, 1996). The upper limit of CO_2 for salmonids set by The Norwegian Food Safety Authority is 15 mg/ (Noble et al., 2018). However, a recent study by Khan et al. (2018) claims that there are no concentration of CO_2 where Atlantic salmon in fresh water are not negatively affected.

As previously mentioned, pH affects the concentration of both CO_2 and unionized ammonia in the water. The pH range that minimizes the detrimental fractions of both ammonia and CO_2 is ≈7.5-8.2 (Summerfelt, 1996). A recommendation is to keep pH in the lower bounds of the optimal range for the nitrifying bacteria (7.0-9.0) to maintain nitrification while minimising effect of ammonia (Noble et al., 2018). To prevent rapid changes in pH, it is important to control the alkalinity of the water.

Alkalinity is a measure of a solutions capacity to neutralize acid, and a level of at least 50 mg/L (as calcium carbonate, $CaCO_3$) is recommended to ensure stable pH (Summerfelt, 1996), although levels up to 100-150 mg/L has been recommended for intensive fish cultivation (Wedemeyer, 1996).

1.2.4 Particles

Particles in the water can be divided into several categories, depending on size and what they are consisting of. Those in the smallest size range are often referred to as fine particles (< 20µm) and colloids (<10µm). In RAS, particles can originate from the intake water, as well as from uneaten feed, fish faeces and sloughed microbial cell masses (Chen et al., 1993). The design of the biofilters can also affect particle levels (Fernandes et al., 2017). It is important to control the presence of particles, as decomposition can degrade water quality, directly or indirectly affect fish performance, and in addition affect other processes within the RAS (Chen et al., 1993).

High levels of organic matter will increase the number of heterotrophic bacteria, leading to reduced oxygen levels and increased ammonia and CO_2 production. Large amounts of particles can also cause sedimentation in areas with low circulation, potentially making anoxic areas where highly toxic hydrogen sulfide (H_2S) may be produced by microorganisms (Wedemeyer, 1996). Particles will also affect the turbidity, how much light penetrates the water, and may therefore reduce the disinfection efficiency of UV-light (Wedemeyer, 1996, Hess-Erga et al., 2008), which is sometimes used as part of the water treatment for biosecurity reasons. In addition, particles can cause physical damage to gills (Chapman et al., 1987), reduce biofilter nitrification rates (Zhu and Chen, 2001), and they have been linked to occurrence of bacterial gill disease and amoeba gill infestation (Bullock et al., 1994).

Particles in water can be measured in different ways, as there is a wide variation in size, what they consist of, and shapes which might be of interest. To measure total amount of particles, methods such as measuring total suspended solids (TSS) and turbidity can be used. Turbidity gives an indication of the total amount of substances that affect the ability of light to penetrate water, while TSS on the other hand, involves determining how much particulate matter above a certain size is present in the water.

A report on how to evaluate and document fish welfare, concluded that there was not enough scientific evidence to set a guideline for optimum level of turbidity or TSS for Atlantic salmon in RAS (Noble et al., 2018), but it has been recommended to keep TSS at a concentration below 15 mg/L (Thorarensen and Farrell, 2011).

1.3 Membrane filtration

Membrane filtration is based on using a semipermeable membrane as a barrier to control which molecules passes through. There are several categories of membrane filtration allowing for removal of constituents in the water based on size, such as microfiltration (≈1.0μm–10μm), ultrafiltration (≈0.01μm–1.0μm), nanofiltration (≈0.001μm–0.01μm) and reverse osmosis (≈0.0001μm–0.001μm) (Chiam and Sarbatly, 2011). Pressure-driven filtration, using hydraulic pressure which forces water through the membrane while other substances are retained, is most common (Chiam and Sarbatly, 2011). Application of membrane filtration is currently not common in aquaculture, but has been utilised in several other industries (Chiam and Sarbatly, 2011, Lekang, 2013). A possible explanation is the high maintenance requirements of

membrane filters due to fouling, causing dramatic reduction in water flow (Lekang, 2013). It is also not regarded as a cost-effective alternative to microscreen filters for coarse solids removal (Viadero and Noblet, 2002), indicating that it might only be economically viable in niche applications, such as in very high-intensity RAS where accumulation of small particles becomes a problem.

While alternative methods for the removal of accumulating particles exists, membrane filtration has several advantages, such as being space efficient, requiring no chemicals and potential disinfecting properties if the pore size is small enough (Lekang, 2013). Previous studies on using membrane filtration in aquaculture indicate positive effects on removal of particles and suspended solids (Holan et al., 2013, Holan et al., 2014), water quality and fish performance of cod larvae (Holan et al., 2014), and changing the composition of microbial communities (Wold et al., 2014). However, there is limited knowledge on the use of membrane filtration in production of Atlantic salmon in RAS.

It has been stated that an increased understanding of the interactions between the fish and the system help facing the challenges of accumulation of substances (Martins et al., 2010). This should be kept in mind when assessing water treatment technology which aims to improve and intensify production.

1.4 Experimental aims

This thesis was associated with the research collaboration project RAS-ORGMAT (RAS-ORGMAT, 2016–2018, ERA-NET COFASP), which aims to develop new strategies and water treatment technologies for removal of particulate organic matter (POM) in land based closed containment recirculation systems for aquaculture, and investigate how different removal rates of organic matter affects carrying capacity of bacteria, off-flavour compounds and waste products.

The aim of this thesis was to investigate the effects of utilising a membrane filter for particle removal on water quality development and fish performance, when rearing Atlantic salmon in RAS. Two pilot-scale RAS were compared; one system using conventional water treatment components (cRAS), and one modified to include a membrane filter (mRAS). Development of selected water quality parameters was monitored, performance of subsamples of the fish

population in each RAS was assessed at selected points during the experiment, and average water quality and fish performance of all fish at the end of the experiment was compared. The results were used to answer whether the utilisation of membrane filtration caused discrepancies to occur between the two systems in 1) the development of selected water quality parameters; and 2) the performance of Atlantic salmon. Ultimately, these answers were combined to determine whether membrane filtration can be said to be beneficial for rearing of Atlantic salmon in RAS, from a water quality and fish performance perspective.

2 Materials and methods

The experiment was conducted at NTNU Centre of Fisheries and Aquaculture (Sealab), Trondheim, Norway, in collaboration with the research project titled "Developing water treatment technology for land-based closed containment systems (LBCC–RAS) to increase efficiency by reducing the negative effects of organic matter" (RAS-ORGMAT, 2016–2018, ERA-NET COFASP). Experimental design and methods for water quality measurements, as well as sampling frequency and sample sizes for fish performance was determined by the project, RAS-ORGMAT. The main experiment was performed using two pilot-scaled recirculating aquaculture systems (RAS), each consisting of 6 fish tanks connected to a water treatment system. One system used conventional water treatment components (cRAS), the other consisting of the same components but modified to include a membrane filter (mRAS). In addition, fish tanks connected to a separate flow-through system were used in tests associated with fish performance. An overview of the experimental setup is presented in Fig. 1.

2.1 Water treatment

Water from the fish tanks arrived in a sump (S1) where make-up water was also added. The water was then filtrated through a drum screen filter (HEX, CM Aqua Technologies, Denmark) with mesh pore size ≈60μm in cRAS and ≈20μm in mRAS, before entering a second sump (S2). From S2, the water went through a moving bed biofilter (Nofitech, Norway) consisting of three consecutive chambers (250L each) filled with biofilm carriers (Nofitech, Norway) with a total surface area of approximately 100 m^2 in each chamber. Prior to stocking of fish, the biofilters were matured by supplying ammonium chloride (NH_4Cl) and fish feed. Biofilters were continuously operated with upstream air and water supply from the bottom throughout the experiment. Upon exiting the biofilters, the water went through a water-to-air counter-flow system for aeration and CO_2-degassing, then collected in a third sump (S3) where oxygen was added, before returning to the fish. In the mRAS, 10% of the water flow was filtrated through an ultrafiltration membrane (X-flow COMPACT 4.0G Ultrafiltration Membrane, Pentair, Netherlands) during the experiment. The membrane filter had two series-connected membrane with areas of 4 m^2 and pore sizes of ≈30 nm, operated with a transmembrane pressure of approximately 0.2 bar. Each RAS had a total water volume of 3500L, and water was transported through the system by gravitation and pumps (Grundfos, Denmark).

Until the start of the experiment, valves connecting each set of sumps were open, causing a constant mixing of water between the systems in S1, S2 and S3, making the two systems operating as one system. At the start of the experiment, the recirculation systems were divided by closing the valves, each recirculation unit treating the water from 6 of the tanks.

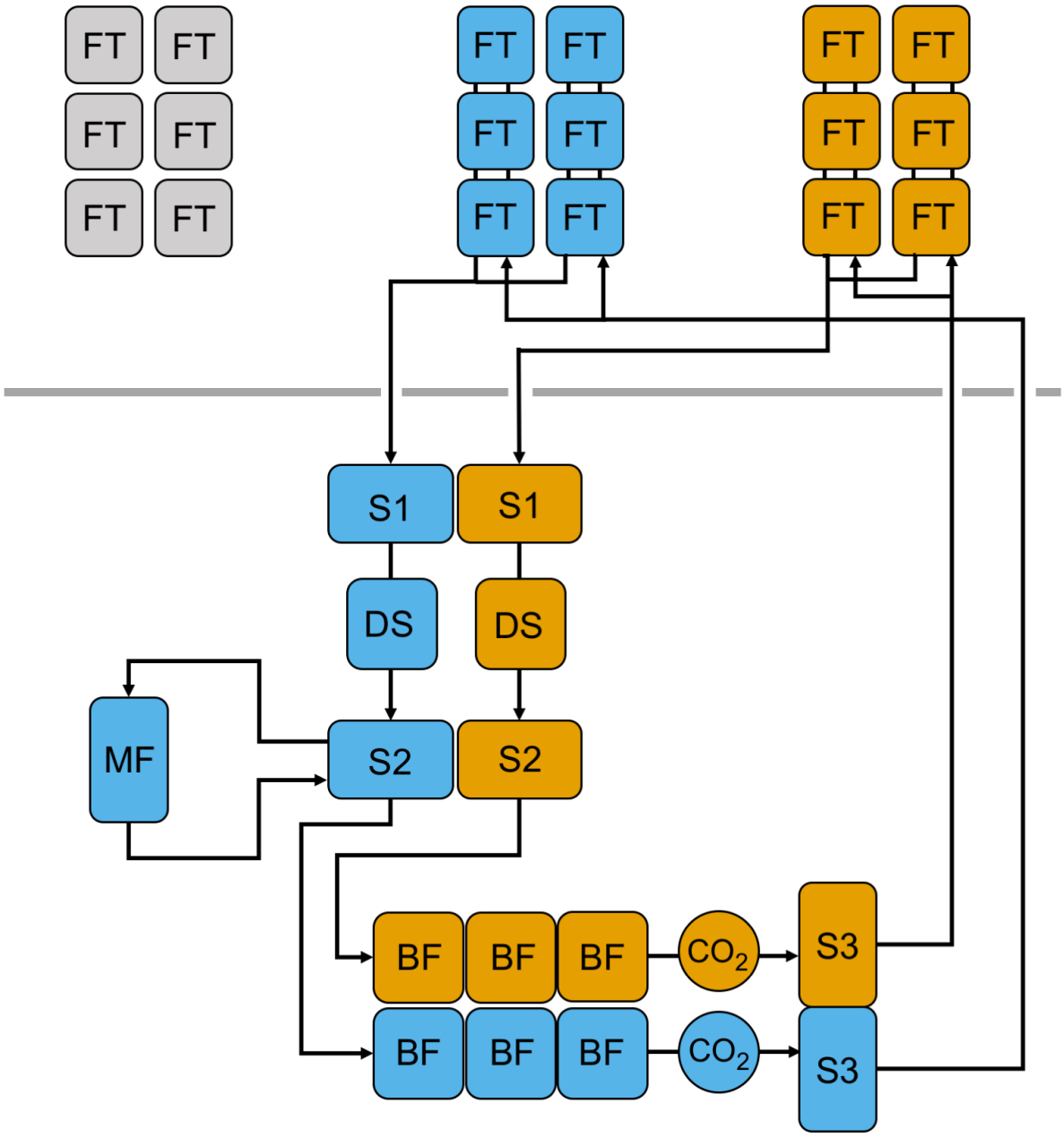

Fig. 1 A schematic overview of the experimental setup, with the room with recirculation treatment components (bottom) and the room with fish tanks (top). Arrows indicate direction of water flow. Both cRAS (orange) and mRAS (blue) consisted of six fish tanks (FT), three sumps (S), a drum screen filter (DS), a biofilter with three chambers (BF) and a degassing unit for removal of carbon dioxide (CO_2). In addition, mRAS had a membrane filter (MF). Six fish tanks (grey, FT) were part of a separate flow-through system, only used for recovery after handling stress tests (Chapter 2.3.2) and for a seawater tolerance test (Chapter 2.3.3).

2.2 Rearing conditions

Atlantic salmon parr (*Salmo salar*) from Marine Harvest Slørdal arrived 24.01.17. Fish were randomly caught using a dip net, and counted when transferred into a large bucket filled with water until there were 60 fish in the bucket. The bucket with water was weighed before and after fish were added to measure total weight of the 60 fish ($W_{0\text{-Tank}}$), then the fish were transferred into one of the fish tanks (0.4m^3, Nofitech, Norway) used in the experiment. This was repeated until all 12 tanks used in the experiment contained 60 fish. Mean weight of fish and tank density per system at stocking was calculated based on $W_{0\text{-Tank}}$, and is presented in Table 1.

Table 1 Average starting weight of fish and mean biomass density per system at stocking.

System	$\overline{W_{o-System}}$ (g)	Mean density (kg/m^3)
cRAS	45.01	6.79
mRAS	46.40	7.17

The fish were given 13 days of acclimatization, with feeding starting the 4. day after stocking, before experiment started at 06.02.17. The experiment lasted 127 days from February until June in 2017 (06.02.17–13.06.17).

The fish were reared with an artificial winter light regime (8L:16D) the entire experimental period. During the light period, feed (3–3.5 mm, Nutra Advance RC, Skretting, Norway) was supplied by automatic feeders (Arvo-Tec Oy, Finland) every 20 minutes. Daily feeding load was approximately 2% of estimated total biomass in the systems to ensure a high load of particles in the systems, down or up-regulated throughout the experiment based on requirements of overfeeding to achieve the wanted experimental water quality conditions.

During a post-experiment count of the total number of fish removed from the tanks, an uneven distribution of fish per tank was revealed. This indicates imprecise counting of fish at arrival, or erroneous handling of fish at end sampling causing some fish to be counted several times or not at all. As the latter could not be proven or adjusted for, it is assumed that the initial number of fish per tank was not exactly 60, which is corrected for in affected calculations, and listed in Table 11 (Appendix I).

2.3 Fish sampling and analyses

Sampling methods and analyses are described in the following sections. Time of sampling, sample sizes, and what is sampled for is listed in Table 12 (Appendix II). Sampling and measurements at week 15 of the experiment was performed by Trond Rosten and Anette Voll Bugten. All other fish samplings and measurements were performed by the author, Anette Voll Bugten, and either Trond Rosten or Carolyn Rosten.

2.3.1 Sedation, euthanizing and blood sampling

A 1:10 dilution of AQUI-S was prepared, and 16ml of this was added to a bucket with 20L of water from a RAS. A second bucket was prepared with 1mL of the diluted AQUI-S and 10L water from the system. Fish were then gently transferred from experimental tanks with a dip net to the bucket with high concentration of anaesthetics for 1 minute or until unresponsive to a pinch in the tail, before they were moved over to the bucket with low concentration to maintain sedation until euthanisation. While fish were in the bucket with low concentration, an aeration pump was added to maintain oxygen saturation in the water.

Anesthetized fish were euthanized by a blow to the head, immediately followed by blood sampling from the caudal vein using a heparinised syringe (1 mL, with needle, 21G 1"; 0.8 x 25mm). The blood sample was transferred to an Eppendorf tube (1.5 mL), then centrifuged for 5 minutes in a VWR Galaxy mini centrifuge. Plasma was transferred to another Eppendorf tube (1.5 mL) using a pipette, then quickly frozen and stored at -80 °C.

Sedation, euthanizing and blood sampling were performed the same way for all baseline samplings, handling stress and seawater tolerance tests.

2.3.2 Recovery from handling stress test

Three fish were removed from a tank with a dip net, one of which was put in sedatives (0h-group). The other two fish were exposed to air for 1 minute before being transferred to either a tank for a 1-hour (1h-group) or a 6-hour (6h-group) recovery period. This was repeated for all the 6 tanks in one RAS before euthanizing and blood sampling of the 0h-group, then the same procedure was repeated for all the 6 tanks in the other RAS. Following sedation, euthanizing and blood sampling was performed on the 1h-groups 1 hour after handling stress, and on the 6h-groups 6 hours after handling stress. Recovery tanks were part of a separate freshwater flow-through system. Oxygen levels and temperatures in the water during recovery in Table 2.

Table 2 Water temperature (°C) and oxygen saturation (%) in outlet of fish tanks during the recovery periods, after the fish experienced handling stress in the form of air exposure during transfer between two tanks using a dip net.

	mRAS		**cRAS**	
Recovery period	1h	6h	1h	6h
Oxygen (%)	96–98	97–99	95–98	97–99
Temperature (°C)	7–8	7–8	7–8	7–8

2.3.3 Seawater tolerance test

A 24h-seawater tolerance test was performed on 6 fish from each system (1 from each tank) at the end of the experiment. The fish were transferred to two flow-through tanks (0.4m^3, all from mRAS in one tank and all from cRAS in the other) with seawater (32.3 ppt, 99% oxygen saturation, 9.5°C in outlet of fish tanks). After 24 hours, the fish were sedated, euthanized and sampled for blood. Half of the plasma was frozen and transported to Marine Harvest Slørdal, where a chloride titration was performed to determine blood chloride levels. The other half was analysed as described in Chapter 2.3.4.

2.3.4 Blood sample analysis

Blood plasma was thawed and analysed for sodium, chloride, potassium, glucose and lactate using an automated analyser (RX daytona, Randox Laboratories Limited, United Kingdom).

2.3.5 Morphology

2.3.5.1 External examination

A visual inspection was performed on all sampled fish, where any signs of damage were quantified according to a scoring index explained in Table 3. Fins, eyes, opercula, gills, snout and mouth were particularly closely investigated. Other deviations from normal morphology that could indicate reduced welfare were noted, but not given a score.

Table 3 Explanation of scoring index used for determining status of damage to external morphological features.

4	3.5	3	2.5	2	1.5	1
No sign of damage	Slight damage, healed or mostly healed	Slight damage	Easily noticeable damage, healed or mostly healed	Easily noticeable damage	Severe damage, healed or mostly healed	Severe damage

Due to limited time, a simplified version of the index was utilized at termination of the experiment, where all fish remaining in the system were scored as either having sustained damage or not (score ≤ 3 or score ≥ 3.5, respectively, as explained in Table 3) to selected external morphological structures.

2.3.5.2 Smoltification indicators

On each sampling, morphological indications of smoltification (body silvering, parr markings and blackening of fin margins) was monitored and given a score on 1–4, where 1 corresponded to parr and 4 to fully smoltified. The smolt index was calculated by taking the average score of these parameters per fish.

2.3.5.3 Internal examination

The final inspection of the fish was to open the abdominal cavity. The state of the internal organs was compared between fish from each system, with special focus on liver, spleen, digestive tract and the amount of fat tissue. It was determined whether colour, shape and size of organs were within normal ranges.

2.3.6 Growth

After blood sampling, wet weight (W, (g)) and fork length (L, (cm)) was measured. Using Eq.1 (Bolger and Connolly, 1989), Fulton's condition factor (K) of sampled fish was calculated.

$$Condition\ factor = \frac{W}{L^3} \times 100 \qquad \text{[Eq. 1]}$$

At the end of the experiment, total weight gain (kg) in each system was calculated using Eq. 2,

$$Total\ body\ weight\ gain = (Weight\ of\ all\ sampled\ and\ dead\ fish) - (Total\ W_0) \quad \text{[Eq. 2]}$$

with total W_0 being the sum of measured total weight of fish per tank ($W_{0\text{-Tank}}$) in each system. Total weight gain was used with the weight of total feed fed to calculate feed conversion rate using Eq. 3.

$$FCR = \frac{Total\ feed\ fed\ in\ period\ (kg)}{Body\ weight\ gain\ in\ period\ (kg)} \quad \text{[Eq. 3]}$$

Specific growth rate (SGR = (% body weight gain/day)) was calculated according to Eq. 4 (Hopkins, 1992), with W_t being wet weight of each fish at the end of the experiment, $\overline{W_{o-System}}$ the average wet weight of the fish in each system at stocking and t the duration of time from the fish arrived until end of experiment.

$$SGR = [\frac{lnW_t - ln\overline{W_{o-System}}}{t}] \times 100 \quad \text{[Eq. 4]}$$

To estimate the effect of temperature on growth, the thermal growth coefficient was calculated with Eq. 5, with T being the average water temperature (°C) in each system from the fish arrived until end of the experiment.

$$TGC = [\frac{\sqrt[3]{W_t} - \sqrt[3]{\overline{W_{o-System}}}}{T \times t}] \times 1000 \quad \text{[Eq. 5]}$$

2.4 Water quality sampling and analyses

Water quality parameters were measured and analysed solely by the author (turbidity, total suspended solids), by the author and other people involved in RAS-ORGMAT (temperature, oxygen, carbon dioxide, salinity, pH and alkalinity) or solely by RAS-ORGMAT (total ammonia nitrogen, nitrite and nitrate). Other water quality parameters were also measured by RAS-ORGMAT, such as dissolved organic matter (DOC), total organic carbon (TOC) and particle size distribution, but are not further discussed in this thesis. Results regarding these parameters and microbial carrying capacities in the systems are discussed by Nesje (2018).

2.4.1 Turbidity

A 2100AN Laboratory Turbidimeter (Hach, USA), was used to measure turbidity (nephelometric turbidity units, NTU) in water samples from sample point S1, S2 and S3 twice a week during the experiment.

The water sample was carefully shaken to make it homogenous without creating air bubbles, before being added up to the indicated level in a clean cuvette. The cuvette was inserted into the instrument for 10–15 seconds before reading the value. This was repeated with new sample 3 times for each sample point. The cuvette was cleaned between each new sample point.

Only measurements from S3 are included in the results, as this was determined to best represent the turbidity experienced by the fish.

2.4.2 Total suspended solids

Total suspended solids (TSS) were measured in water samples from S1, S2 and S3. Glass fibre filters (1.2 micron) in aluminium containers were weighted to determine the start weight (W0), and placed in a desiccator. 200mL of sample (V) was measured for filtration. A filtering apparatus consisting of a vacuum cylinder, filter holder and sample cylinder was used to filter the sample. The filter was placed on the filter holder with tweezers, and the filter holder put on the vacuum cylinder. The sample cylinder was attached on top of the filter holder, using a clamp to keep all components in place. Then the vacuum was put on by mounting vacuum tubing to the vacuum cylinder. Milli-Q water was used to flush the filter, before sample was filled in the sample cylinder. After the sample was filtered through, the sample cylinder was carefully and thoroughly flushed with milli-Q water, the clamp removed, and then the filter holder was flushed. Vacuum tubing was removed, and the filter transferred back to the aluminium container using tweezers. Filter and container was placed in a preheated oven (105°C) for drying until a stable weight was achieved. When dry, filter and container was removed and placed in a desiccator for cooling, before weighted (W1).

Based on the initial weight of the filter (W0), the end weight of the filter (W1) and the volume of filtrated sample (V), the TSS was calculated according to Eq. 6.

$$TSS = \frac{W1-W0}{V} * 1000\ [\frac{mg}{L}] \qquad \text{[Eq. 6]}$$

The filtrated sample volume was increased to 500 mL from 17.02.2017 to improve precision of the measurement. Due to time and budget limitations, TSS sampling and analysis was terminated after a final sampling on 24.02.17 by request of those responsible for TSS in RAS-ORGMAT.

2.4.1 Temperature, oxygen and salinity

The water temperature, oxygen saturation and salinity was measured daily in the outlet of the fish tanks using a Pro2030 handheld dissolved oxygen meter (YSI, USA). Temperature regulation was primarily done by adjusting room temperature in the fish hall. Due to diverging temperatures in the two systems, and periodic incidents of too high temperatures in mRAS, a cooling coil was placed in S2 in mRAS in week 7 of the experiment.

2.4.1 Total ammonia nitrogen, nitrite and nitrate

Water samples from S3 was analysed for total nitrogen ammonia (TAN, NH_3+NH_4^+), nitrite (NO_2^--N) and nitrate (NO_3^--N) 1-2 times a week using a DR/890 Colorimeter (HACH, USA) and methods 8155, 8507 and 8039. Measured twice a week, 2-3 replicates.

2.4.2 Alkalinity, pH and CO_2

Water was sampled at S1 and S3 5-7 times a week. 100 mL of sample was poured into a beaker with a magnetic stirrer and a pH meter, and pH was measured. On samples from S3, titration with grade HCL 0.1 N was performed until end-point of pH 4.5 was reached. Volume HCl was used to calculate total alkalinity (mg $CaCO_3$/L) using Eq. 7. When necessary, sodium bicarbonate ($NaHCO_3$) was added in the sumps to maintain an alkalinity of ≈50 mg $CaCO_3$/L in the systems.

$$Total\ alkalinity = \frac{volume\ HCl \times 0.1 \times 50000}{volume\ sample\ (100\ mL)} \qquad \text{[Eq. 7]}$$

Dissolved CO_2 (mg/L) was measured with a dissolved CO_2 analyser (Oxyguard, Denmark) in S3 (Fig. 1) during the experiment.

2.5 Statistics

All statistical procedures were performed using the statistical software R. All tests were done at a significance level of p = 0.05, with *p*-values of performed tests listed in tables and figures in Chapter 4.

Weight, length, condition factor, SGR and TGC data from each system was tested for normality using a Shapiro-Wilks test, which yielded strong indication that only SGR and TGC followed a normal distribution. SGR and TGC factor were tested for difference in mean using a Welch

two-sample *t*-test (Welch, 1947), while a non-parametric Mann-Whitney-Wilcoxon test was performed on weight, length and condition factor data to determine if the populations from the systems were identical.

For external morphology, a test of Chi-square on a 2x2 contingency table was performed to test if there was a significant difference in proportion of scores between systems. For blood values from handling stress tests, a Mann-Whitney-Wilcoxon test was performed on each time point before and after recovery to investigate population differences between cRAS and mRAS.

For water quality parameters, all measurements from the experimental period from each system are treated as the sample populations. Shapiro-Wilks test for normality was conducted to decide whether populations followed a normal distribution. As all populations for all water quality parameters indicated non-normality, a non-parametric Mann-Whitney-Wilcoxon test was performed to decide if the populations were significantly different.

3 Methodological considerations

The experiment was intentionally designed to have higher than normal particle loads on the system. If no differences were to be uncovered, it would be safe to say that no differences would occur under normal rearing conditions of Atlantic salmon. Due to technical difficulties with operating the systems, and issues with overloading of organic matter requiring adjustments to the operational conditions to be able to continue the experiment, the experimental duration can be divided into 5 distinct periods with different operational regimes which are likely to have affected water quality and fish performance. These were the 5 periods:

Acclimatisation (A; weeks -2 and -1): The acclimatisation period lasted from stocking of fish until the membrane filter in mRAS was initiated, with the purpose of letting the fish acclimatise to the environment. During this period, drum filters were flushed with system water and the systems were run as one system.

Period 1 (P1; weeks 0-5): P1 was the first period where the membrane was operational, where the two systems were supposed to be separated. The biofilters received different particle loads, but water from both systems were slightly mixed in the S3 (Fig. 1) due to a leak, before entering fish tanks. Fish tanks had to be flushed regularly due to settling of uneaten feed pellets at the bottom of the tanks.

Period 2 (P2; end of week 5 to end of week 10): A leak was discovered and immediately closed, and a high degree of recirculation was maintained the first half of the period. As the amount of particulate matter in the water increased, more water was lost with sludge leaving the drum screen filters, requiring more water to be added and lowering the degree of recirculation.

Period 3 (P3; weeks 11-14): Due to rapidly deteriorating water quality in P2, flushing of drum filters were changed to use new water at onset of P3. Any other source of make-up water was stopped, but frequent flushing of drum filters resulted in decreased recirculation. Feeding was periodically downregulated to decrease particulate load on the systems.

Period 4 (P4; weeks 15-18): For the final period of the experiment, operation of mechanical filters was changed back to use system water for flushing and sensitivity for flushing adjusted.

In addition, the mesh size of the drum screen filter in mRAS was changed from 20µm to 60µm, which was what was used in cRAS. The degree of overfeeding was continuously increased throughout the period.

These procedures were performed the same way in both systems to ensure that within each period, the capacity to remove particulate matter was the variable differentiating mRAS from cRAS. The changes in operational conditions combined resulted in 2 periods of low particulate load on the systems (P1 and P3) and 2 periods with high particulate load (P2 and P4) within the duration of the experiment. All figures displaying development of water quality and fish performance have background colours which reflect the respective period, as shown in Fig. 2.

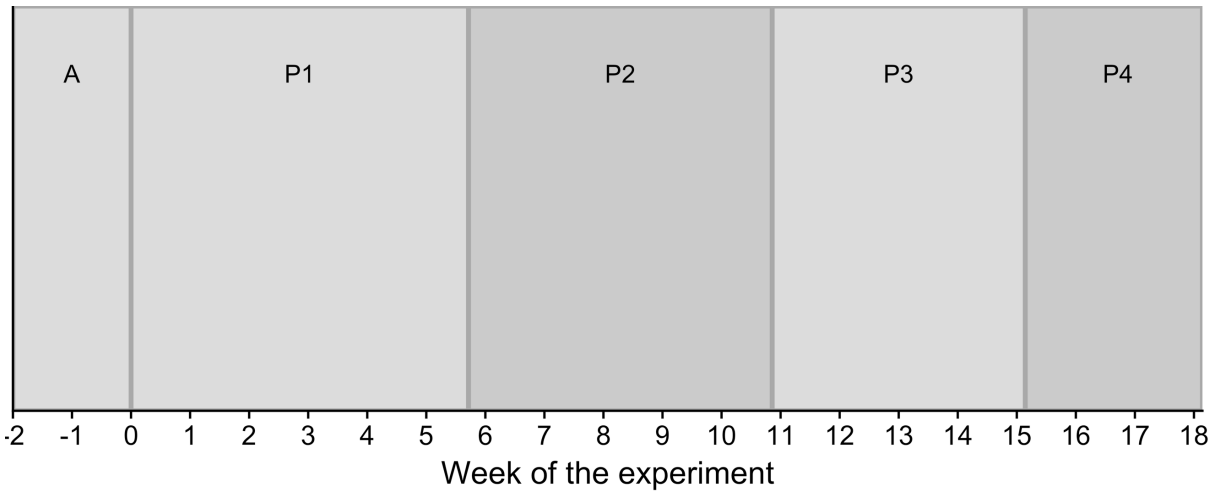

Fig. 2 Periods within the experiment with low (light grey) and high (dark grey) particulate load on the systems. Start and end of each period (A, P1, P2, P3 and P4) is based on dates, while displayed labels on the x-axis are weeks of the experiment.

As these changes were not a part of the preliminary experimental design, the initial planned sampling dates for fish performance parameters did not coincide well with the start and end of the periods of different load, making it difficult to link changes in performance to specific periods. In addition, there were issues with coagulation in most of the blood plasma samples, making further analysis difficult and results unreliable. When noticed, a droplet of heparin was added to the thawing plasma and the sample was centrifuged prior to analysis. There was not sufficient reliable data to establish development of baseline values, nor a full analysis of blood from handling stress and seawater tolerance tests.

4 Results

The figures visualising development of water quality parameters (Chapter 4.1) and fish performance (Chapter 4.2), as well as tables summarising and comparing results are presented in the following chapters.

4.1 Water quality development

The figures of water quality development show all individual measurements performed during the experiment, to visualize major trends as well as variation within weeks of the different periods. A summary of average levels of water quality from the experiment is presented in Chapter 4.1.5.

4.1.1 Recirculation, feed, turbidity and TSS

During the first half of the experiment, both systems had a high and stable recirculation of >90% of the water each day (Fig. 3A). During the second half of the experiment, the daily recirculation was lower and more irregular. In the final period, recirculation was mostly stable at ≈70%. The feeding load per day was kept the same for both systems, with a steady increase up until the middle of P2, with two episodes of downregulation in P3 and a rapid increase during P4 (Fig. 3B). Measurements of turbidity in the systems indicated a small but constant difference during the first weeks of the experiment (Fig. 3C). From the onset of P2 the turbidity in the systems started diverging, with cRAS increasing until the middle of week 11 (from ≈1.0 to ≈6.4 NTU), while mRAS experienced a slight decrease (from ≈0.8 to ≈0.5 NTU) before increasing and peaking at the onset of week 11 (≈1.5 NTU). Turbidity rapidly stabilized in mRAS during P3, compared to cRAS where the turbidity decreased until the start of the next period. Both systems experienced an increase in turbidity in the final period of the experiment, with cRAS reaching higher turbidity than mRAS (≈11 and ≈5 NTU, respectively).

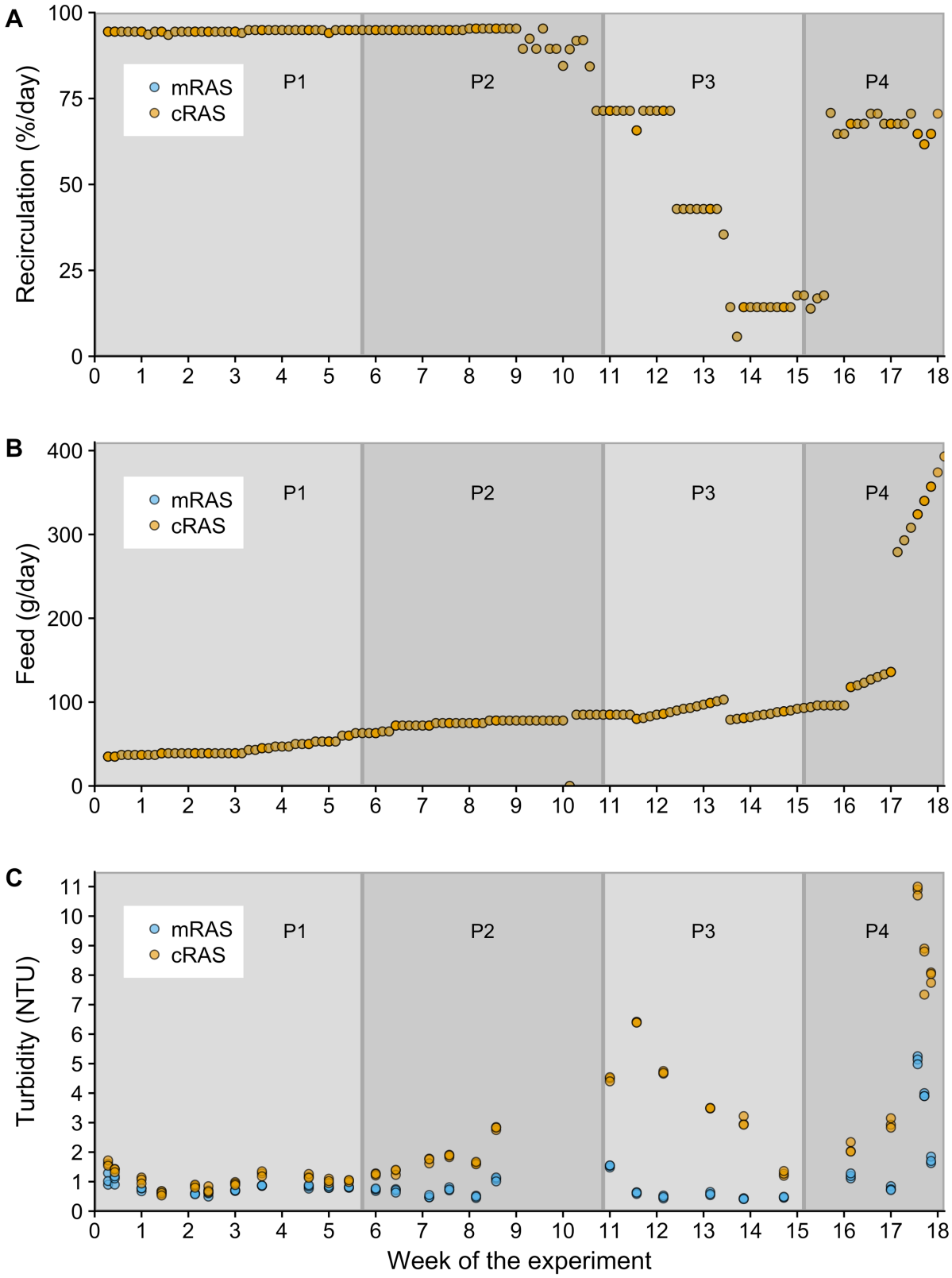

Fig. 3 Development of recirculation and added feed affecting total particulate load in the systems (blue = mRAS and orange = cRAS) throughout the experiment, and the turbidity of the water reflecting the difference between systems capacity to remove particles under different particulate loads (periods P1-P4). **A)** Recirculated water (%/day), equal values for both systems every day. **B)** Amount of feed added per system (g/day), equal values for both systems every day. **C)** Turbidity (NTU) in each system.

Total suspended solids (TSS) was only measured the first 3 weeks. In mRAS it decreased from 2 mg/L to ≈0 mg/L while in cRAS a decrease from 2 mg/L to ≈1 mg/L was measured (Table 4).

Table 4 Measured TSS (mg/L) in water samples from S1 in mRAS and cRAS.

Week of the experiment	mRAS	cRAS
0	2	2
0	2	2
1	0.75	1.2
2	0.6	1
2	0	1.2

4.1.2 Temperature, oxygen and salinity

Throughout the entire experimental period, there was a high variance in measured water temperature, with the difference between systems increasing and decreasing in different periods (Fig. 4A). Temperature rapidly diverged at the onset of P2 until week 7 (peak discrepancy of ≈2.8 °C between mRAS and cRAS), after which temperature in mRAS dropped back down to similar levels found in cRAS (≈13 °C). Measured oxygen saturation was mostly stable around 100% in both systems, with a few clear exceptions (Fig. 4B). In mRAS in week 2, in both systems at irregular periods during P2 and at the end of P3, measurements indicated the fish experienced a high saturation of oxygen in the water (>120%). From the onset of the P2-period and throughout the rest of the experiment, results indicate a higher level of oxygen saturation in cRAS compared to mRAS. Towards the end of the experiment, during P4, measured O_2-levels decreased in both systems. Measured salinity increased the first 3-4 weeks (from ≈5.5 ppt), before decreasing to a mostly stable at ≈6.5 ppt from week 10 (Fig. 4C). The salinity was mostly equal in both the systems, apart from a period of up to ≈1 ppt higher salinity in cRAS during week 12-15.

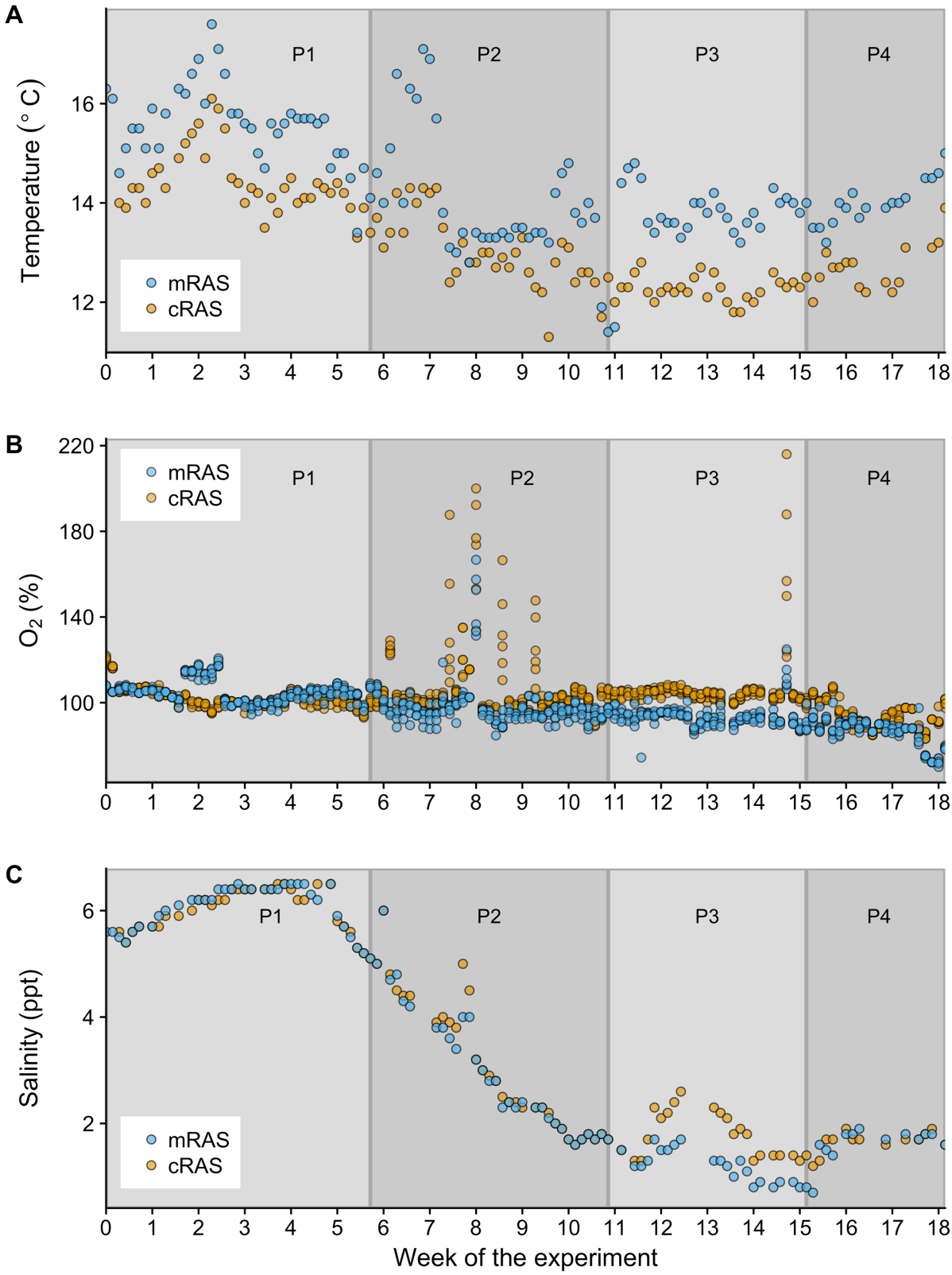

Fig. 4 Measured values of water quality parameters throughout the experiment. **A)** Temperature (°C) measurement in each system. **B)** Oxygen saturation (%) in water from the outlet of each fish tank. **C)** Salinity (ppt) measurements in each system.

4.1.3 TAN, nitrite and nitrate

The peak concentration of TAN was observed during P1 (≈1.3 mg/L in both systems), with low and similar levels in both systems throughout the rest of the experiment (Fig. 5A). A peak of nitrite concentration was also observed in P1 (≈1.2 mg/L in both systems), decreasing in cRAS and mRAS from week 3 (Fig. 5B). A higher variation in both systems was observed for nitrite concentration, apart from decreasing levels during P2 and low levels in P4 (Fig. 5C).

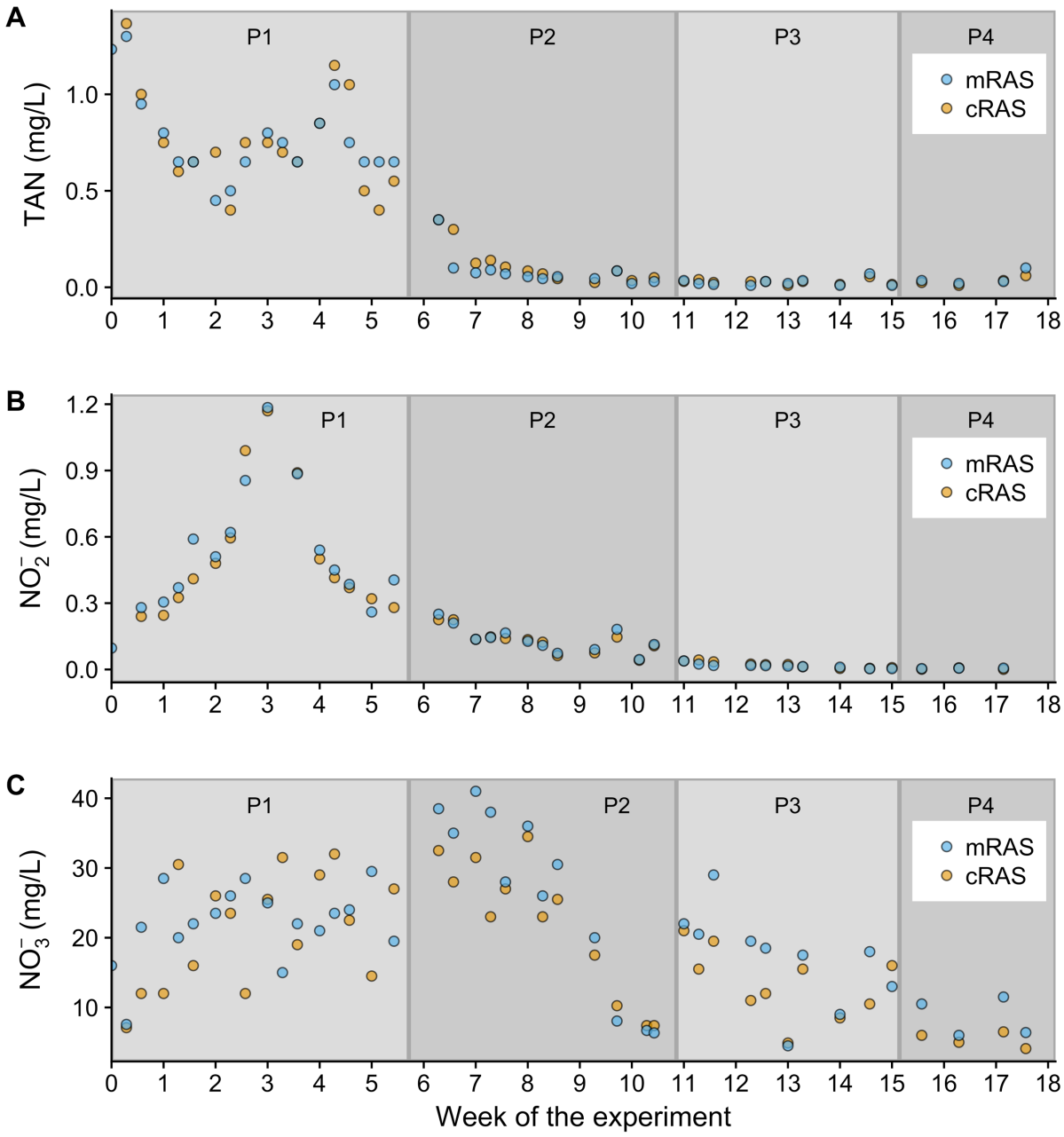

Fig. 5 Concentration of nitrogen compounds in the water after biofilters in mRAS and cRAS during the experiment. **A)** Total ammonia nitrogen (TAN, mg/L). **B)** Nitrite (NO_2^-, mg/L). **C)** Nitrate (NO_3^-, mg/L).

4.1.4 CO_2, pH and alkalinity

The measured concentrations of carbon dioxide were very low in both systems during the entire experiment (Fig. 6A). pH was at similar levels in mRAS and cRAS. Some fluctuation was observed, mostly within the range of 7.5-8, with a noticeable peak during week 3 and drops in the second half of P2 and P4 (Fig. 6B). Total alkalinity experienced a rapid drop from ≈100 down to 50 mg/L during P1, before stabilizing (Fig. 6C).

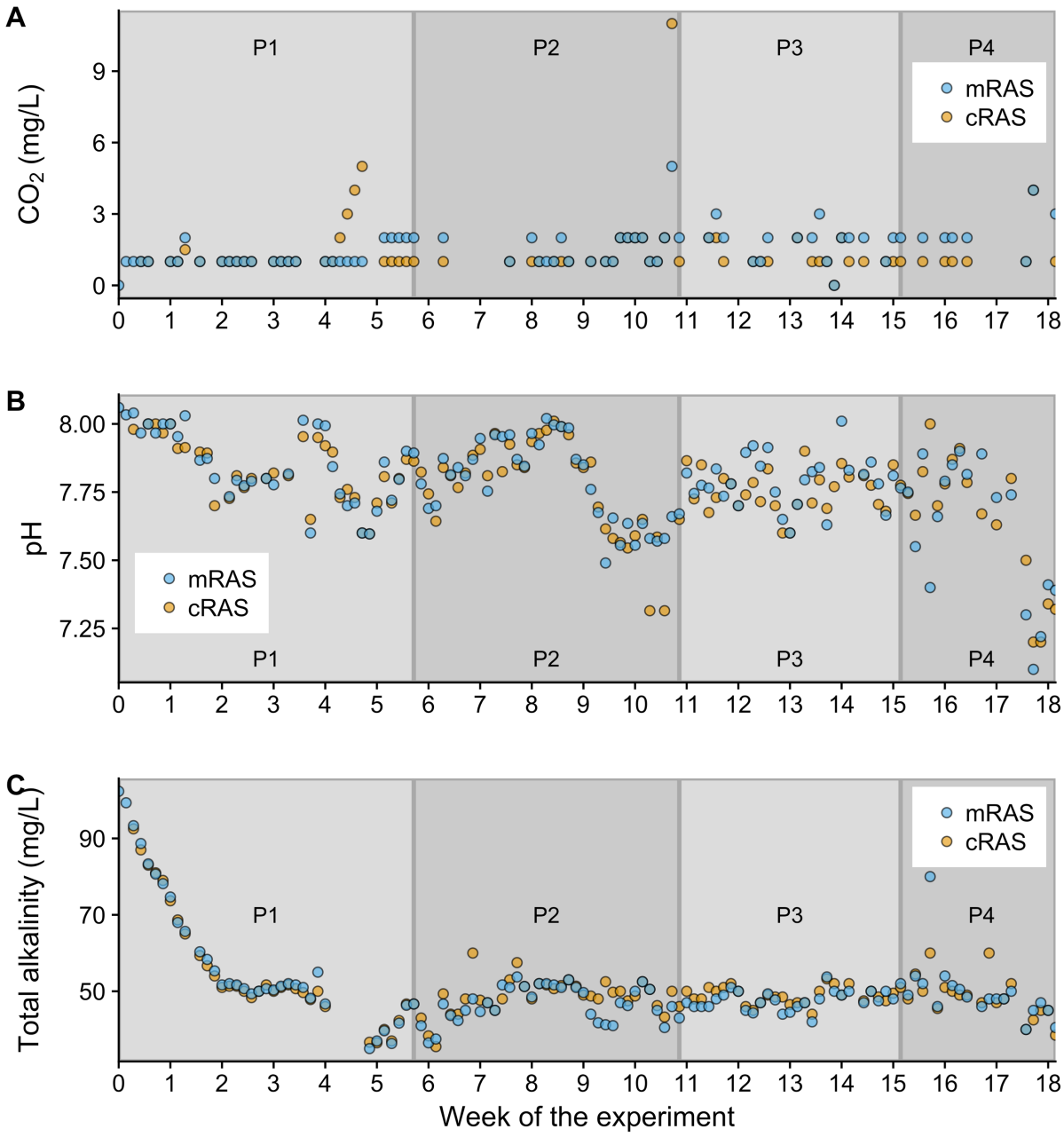

Fig. 6 Development of water quality in both systems during the experiment **A)** Concentration of CO_2 (mg/L) in each system. **B)** pH in each system. **C)** Total alkalinity (mg CaCO3/L) in each system.

4.1.5 Water quality summary

For the entire experimental period, the two systems had overall significantly different mean temperature, turbidity, CO_2- and O_2-concentrations (Table 5). Results for all other water quality parameters and added buffer ($NaHCO_3$) did not indicate significant differences.

Table 5 Average of all measured water quality parameters (mean and SD) during the experiment, as well as mean added $NaHCO_3$ to buffer each system (mean and SD). Significant differences ($p < 0.05$) between systems highlighted with grey.

	mRAS		cRAS		
	Mean	**SD**	**Mean**	**SD**	***p*-value**
Turbidity	1.18	1.03	3.52	2.91	<0.0001
Temperature	14.47	1.19	13.24	1.02	<0.0001
Oxygen	97.21	9.51	103.01	12.09	<0.0001
Salinity	3.50	2.11	3.57	1.96	0.4804
TAN	0.35	0.40	0.34	0.38	0.9830
Nitrite	0.24	0.28	0.23	0.28	0.9609
Nitrate	20.77	9.60	18.18	9.18	0.2756
CO_2	1.55	0.80	1.46	1.41	0.0134
pH	7.78	0.17	7.76	0.16	0.3781
Total alkalinity	50.96	11.72	50.68	9.11	0.3561
$NaHCO_3$	24.30	20.75	21.79	18.50	0.4393

4.2 Fish performance development

Both systems had low mortality with only 3 dead fish in each system during the experiment (<1%). 5 of the total 6 fish died in the first weeks of the experiment (week 0 and 2), and 1 from cRAS died in week 15 of the experiment. FCR for the entire experiment was lower in mRAS (FCR = 2.58) than in cRAS (FCR = 3.27).

4.2.1 Weight, length, condition factor and growth

Weight measurements indicate a decrease in weight from stocking of fish until experiment start (from week -2 to 0, Fig. 7A). Similar growth patterns were observed in fish from both mRAS and cRAS, except for week 0-3, where mRAS seems to have increase more in both weight and length (Fig. 7A and Fig. 7B). An increase in condition factor was observed in both systems during the first 8 weeks (cRAS from 1.12 to 1.24, mRAS from 1.08 to 1.31) (Fig. 7C). During P2, a slight decrease was observed in both cRAS and mRAS (down by 0.05 and 0.07, respectively), followed by mostly stable values until P4. At the end of P4, mean condition factor was similar in both system, but slightly higher in cRAS (1.14 in mRAS, 1.16 in cRAS).

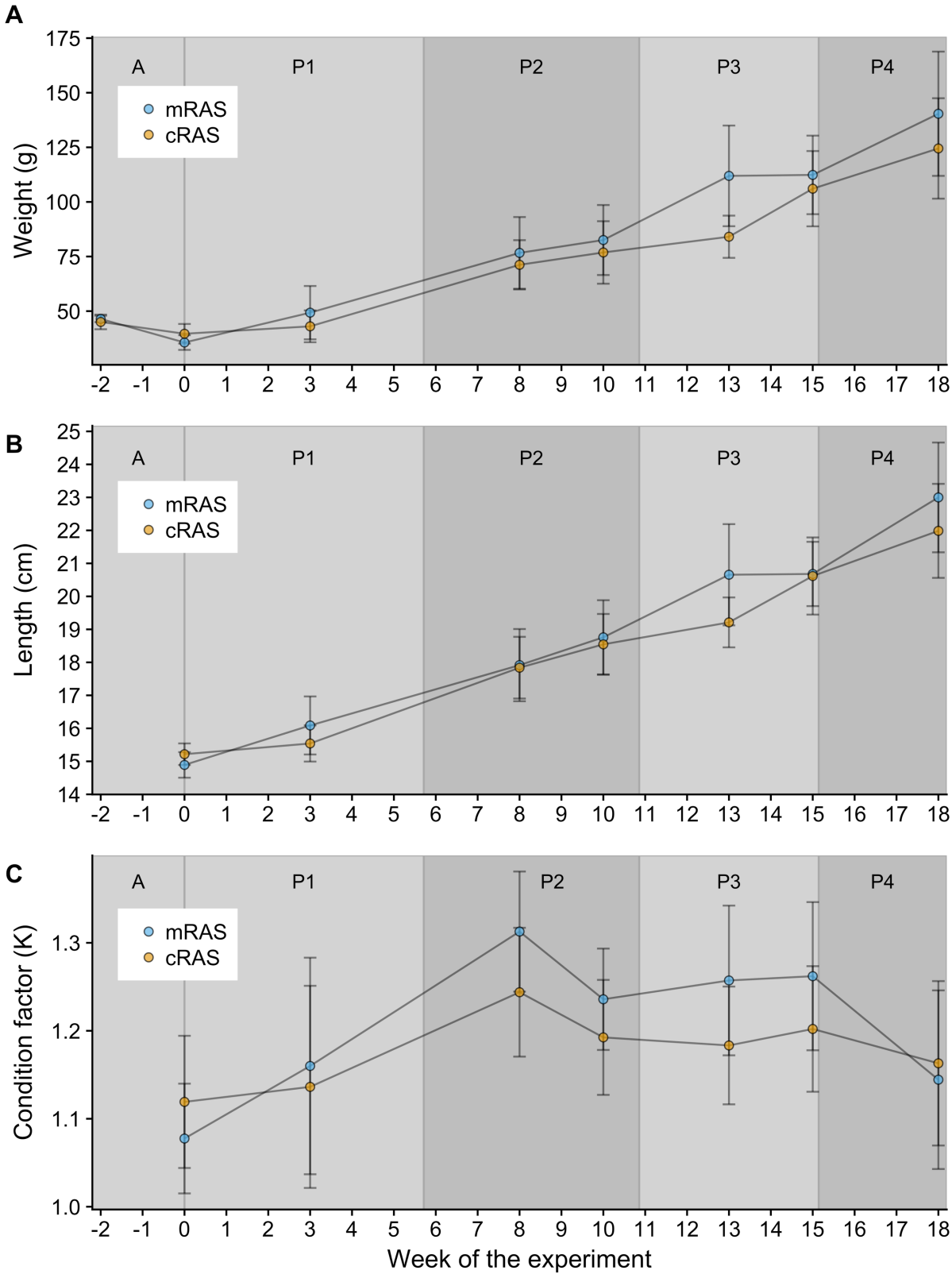

Fig. 7 Weight, length and condition factor (mean ± SD) from all sampling points during the experiment. Data points for weight in week -2 and week 18 is $\overline{W_{o-System}}$ and W_t respectively, used for calculation of SGR and TGC (Table 6). Growth rates within P4 (Table 7) used mean weight per system from week 15 instead of $\overline{W_{o-System}}$.

There was significant difference in growth development between the two RAS, with the fish from mRAS having a higher SGR (Table 6). Fish from mRAS was not only bigger in terms of wet weight, but also in length (Table 6). Condition factor was also significantly different, being 0.02 higher in cRAS. When compensating for the effect of temperature on growth (TGC), the difference in growth was no longer significant (p = 0.08).

Table 6 SGR and TGC from the period 24.01.17-13.06.17. Weight, length and condition factor at the end of the experiment (13.06.17) in mRAS (n = 217) and cRAS (n = 207). Significant differences (p < 0.05) between systems highlighted with grey.

	mRAS		cRAS		
	Mean	**SD**	**Mean**	**SD**	***p*-value**
SGR	0.86	0.16	0.79	0.14	<0.0001
TGC	0.86	0.19	0.82	0.18	0.0800
Weight (g)	140.63	28.80	124.22	22.90	<0.0001
Length (cm)	23.02	1.67	21.96	1.43	<0.0001
Condition factor	1.14	0.10	1.16	0.10	0.0293

The sampling of fish in week 15 and 18 were the only 2 samplings to correspond to the start and end of a high load period (P4), during which there was a significant difference in both SGR and TGC (Table 7).

Table 7 SGR and TGC from the period 22.05.17-13.06.17, in mRAS (n = 217) and cRAS (n = 207). Significant differences (p < 0.05) between systems highlighted with grey.

	mRAS		cRAS		
	Mean	**SD**	**Mean**	**SD**	***p*-value**
SGR	0.93	0.92	0.64	0.83	0.0009
TGC	0.20	0.20	0.15	0.19	0.0081

4.2.2 Morphology

The visual inspection of morphology yielded similar and high mean scores in both systems at all samplings, indicating low presence of severe damages (Fig. 8A). Fins were the only structures to consistently yield an average score less than 4 throughout the experimental period (Fig. 8C). An increase in mean smolt index score was observed from week 3 to week 10, indicating transition from parr to smolt, followed by a slight decrease and another increase towards the end of the experiment (Fig. 8B). The fish had mostly smoltified at the end of the experiment, according to smolt index results.

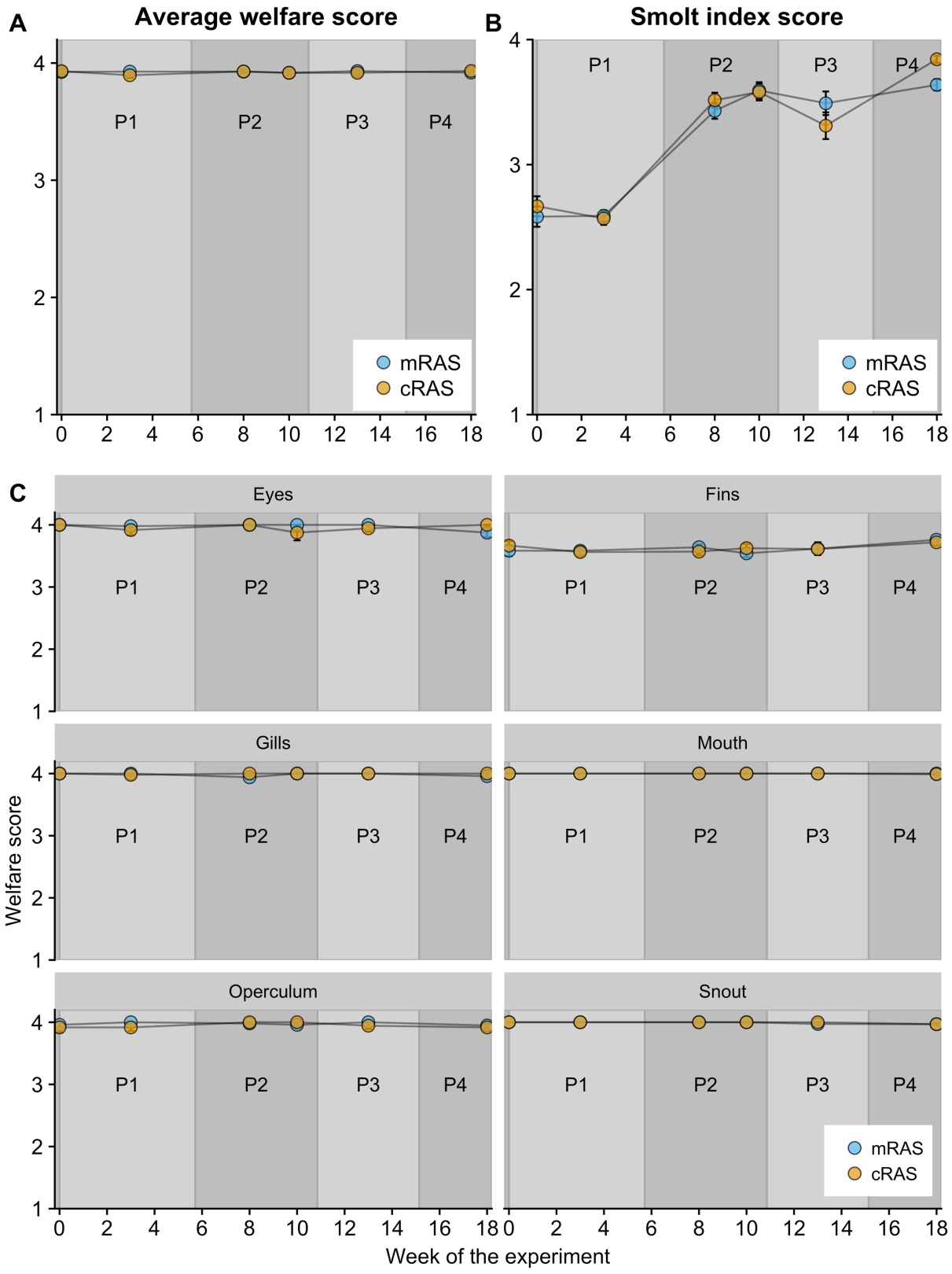

Fig. 8 Score (mean ± SD) from visual inspection of external morphology on fish from mRAS and cRAS from each sampling during the experiment. **A)** Average score from all examined structures combined. **B)** Average smolt index score per sampling. **C)** Average score of each examined structure of external morphology per sampling.

For all fish sampled during the experiment, excluding the final sampling day, no significant difference for scores ≤ 3 was observed between the systems for any of the individual morphological structures investigated (Table 8). For all fish sampled the final day of the experiment, a significant difference in frequency of damage to fins, eyes and the snout was detected (Table 8). When combining occurrence of damage during the experiment with those observed in the final sampling, only fin damage had significantly different occurrence, being higher in cRAS (Table 8). It should be noted that damage to the skin on top of the head, as well as vertebral deformities, was not actively looked for but observed in several fish during the final sampling. Snout damage was actively looked for, but no occurrences were observed prior to final sampling. During the final sampling, gill damage was not investigated.

Table 8 All occurrences of scores ≤ 3 (x) for fish sampled during the experiment (mRAS, n = 151 and cRAS, n = 152), only on the final day (mRAS, n = 217 and cRAS, n = 207), and total combined results (mRAS, n = 368 and cRAS, n = 359). Significant differences between systems highlighted with grey.

		mRAS		cRAS		*p*-value
		x	% of n	x	% of n	
Only during experiment	**Fins**	11	6.29	15	8.52	0.4221
	Eyes	1	0.57	4	2.27	0.1785
	Mouth	0	0.00	0	0.00	NA
	Snout	0	0.00	0	0.00	NA
	Opercula	1	0.57	6	3.41	0.0570
	Gills	0	0.00	2	1.14	0.1573
Only at the end	**Fins**	22	10.14	38	18.36	0.0152
	Eyes	14	6.45	4	1.93	0.0211
	Snout	2	0.92	9	4.35	0.0265
	Opercula	13	5.99	8	3.86	0.3132
	Head	12	5.53	15	7.25	0.4694
	Vertebral deformities	2	0.92	0	0.00	0.1662
Total	**Fins**	33	8.97	53	14.76	0.0212
	Eyes	15	4.08	8	2.23	0.2258
	Snout	2	0.54	9	2.51	0.0623
	Opercula	14	3.80	14	3.90	1

When combining all damage scores from during the experiment into groups based on grade of severity instead of morphological structure, a significant difference in frequency between systems is only observed for the group containing scores equal to or less than 3 (Table 9). End of the experiment samplings were not included in this analysis, as severity of damage was not determined at that sampling, see Chapter 2.3.5.1.

Table 9 Frequency of all scores less than 4 from all except the final sampling (mRAS n = 755, cRAS = 760). Significant differences ($p < 0.05$) between systems highlighted with grey.

Score	mRAS	cRAS	*p*-value
≤ 3.5	95	98	0.8555
≤ 3	13	27	0.0263
≤ 2.5	2	4	0.4179
≤ 2	1	4	0.1814
≤ 1.5	1	1	0.9963
=1	1	1	0.9963

Inspection of internal morphologies did not reveal any discrepancies between the fish from cRAS and mRAS, nor were there any distinct occurrences of deviation from the normal range of colour, size or shape of investigated organs.

4.2.3 Recovery from handling- and seawater tolerance tests

As coagulation occurred in most of the blood plasma samples at some point between sampling and after thawing, there were only limited results of reliable quality. These indicate no significant difference between systems prior to handling (0), 1 and 6 hours after handling, for glucose (Fig. 9 top) and lactate (Fig. 9 bottom). Although no differences were detected between systems, there is a developmental change over time, where neither the fish from mRAS nor those from cRAS have recovered back to resting levels of glucose after a 6-hour recovery period in week 13 and 18, contrary to the fish sampled prior to the start of the experiment (week -1). For lactate, there is a much higher variance in the response 1 hour after handling for mRAS compared to cRAS.

Plasma samples from seawater tolerance test sent to Marine Harvest for analysis, indicate that fish from both systems could osmoregulate in seawater at the end of the experiment (Table 10).

Table 10 Plasma chloride values of fish (mean ± SD, n = 6) prior to seawater tolerance test (0h) and after 24 hours of exposure to seawater (24h).

System	0h	24h
mRAS	122.83 ± 2.32	130.17 ± 5.12
cRAS	122.50 ± 1.64	130.67 ± 2.58

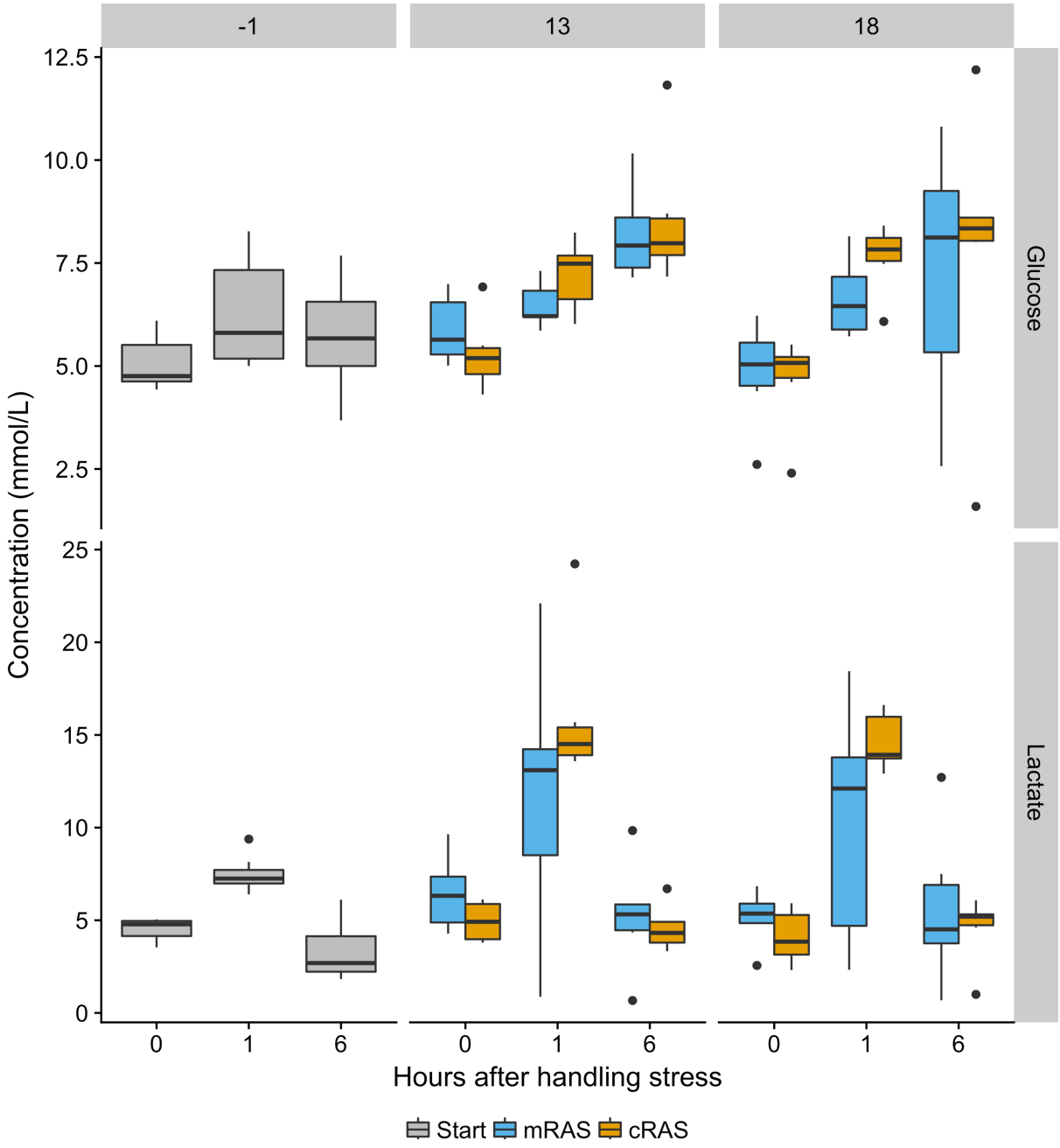

Fig. 9 Boxplot with concentration (mmol/L, y-axis) of glucose (top) and lactate (bottom) at week -1 (left), week 13 (middle) and week 18 (right) of the experiment, prior to (0), 1 hour (1) and 6 hours (6) after handing stress (x-axis), in fish at the start of the experiment (grey boxplots), and in mRAS (blue boxplots) and cRAS (orange boxplots) during the experiment. Mann-Whitney-Wilcoxon tests indicate no significant difference ($p > 0.05$) between mRAS and cRAS at any time point (0, 1 and 6), for any week.

5 Discussion

This study was conducted in collaboration with the research project RAS-ORGMAT. Several other studies were part of the experiment, looking at microbial carrying capacity (Nesje, 2018), microbiota in fish and guts (Master thesis by Anette V. Bugten, unpublished), as well as various technological, economical and off-flavour aspects (unpublished data). My focus was on water quality development and fish performance. Together, the results enable thorough analyses on the many aspects of particle removal in RAS. However, the collaboration limited my influence on how to perform the experiment, and some of the decisions taken during the experiment were not beneficial for studying the effects of membrane filtration on water quality and fish performance. The consequence is that it is difficult to determine when differences in fish performance occurred, and what caused them, since sample sizes and time of sampling of fish did not correspond to changes in operational conditions. In addition, since the 18-week experiment can be divided into distinct periods with different operational conditions (P1 with low particulate load and partial mixing of water between systems, P2 with high load on both systems, P3 with low load on both systems, and P4 with high load on both systems), the results from this study are not purely the outcome of utilising membrane filtration. Therefore, it is beneficial to discuss the causes of the observed developmental trends and correlations, in order to understand the many interactions between rearing regimes, water quality and fish performance in this experiment.

In this study, there were four water quality parameters where mRAS and cRAS displayed significantly different means. These were turbidity, temperature, dissolved oxygen and carbon dioxide. Therefore, the discussion below is structured around these parameters, focusing on possible explanations of the observed differences, how they might have interacted with other water quality parameters, and consequences for fish performance. Lastly, other observations and developmental trends worth noting are discussed.

5.1 Turbidity, and associated effects

In this study, the primary measurement for determining the amount of particulate matter in the water was turbidity. Utilisation of membrane filtration had a clear effect on turbidity, with the mRAS managing to keep turbidity at lower levels than cRAS and stabilising more rapidly after a period of high particulate load (Fig. 3). This shows that the membrane filter is effective at removing particles from the water, which is in accordance with previous studies (Holan et al.,

2013, Holan et al., 2014). Changes in water exchange rates also affected turbidity levels, with turbidity decreasing as the amount of recirculated water decreased. However, these changes in recirculation were similar in both systems, and should not have contributed to the observed differences when comparing mRAS to cRAS. A factor which might have contributed to the observed differences, is the tank design. Despite frequent flushing of tanks, the tank design prevented complete removal of settled uneaten feed pellets near the outlet, causing an accumulation of slowly deteriorating organic matter in the fish tanks, which otherwise would have been removed by the drum screen filters. This amounted for a source of continuous production of particles, and means that even in the periods with low particulate load, especially in P3, there was still quite a high influx of small particles. Given that the feed conversion rate was higher in cRAS than in mRAS, it is reasonable to assume less feed was consumed, and therefore more could have accumulated in the tanks, consequently affecting turbidity in cRAS more than in mRAS.

5.1.1 Effects on other water quality parameters

With increased turbidity, it is reasonable to assume that the total suspended solids (TSS) would increase as well. Knowledge to be gained from TSS results in this study is however limited, since measurements were discontinued early in the experiment. This was primarily due to budget and time limitations, in addition to evidence from particle size analyses indicating that most of the particles in the water was within the smallest detectable fractions (2-3µm) of the method used (Nesje, 2018). Given that the method for measuring TSS used filters with 1.2µm pore size, and the particle size distribution indicated exponential increase in frequency of the smallest detectable particles, it is likely that many of the particles were below the detectable size range. As the TSS results presented in Table 4 were based on measurements from sampling point S1, they were not severely affected by the mixing of water between cRAS and mRAS that occurred during P1. Despite some mixing, and being at an early stage of the experiment, the results suggests TSS in mRAS decreased more rapidly than in cRAS after the membrane filter was activated, and that cRAS showed signs of stabilising at a higher TSS concentration than mRAS.

Changes were observed in other water quality parameters in response to high particulate loads, although these changes displayed similar patterns in both systems. Notable developments include decreases in pH and nitrate during P2 (Fig. 6B and Fig. 5C, respectively), and decreases

in pH, nitrate and oxygen (Fig. 4B) in P4, all correlating with increased particulate load in the system. Decreases in these parameters could be expected in response to high particulate loads, as increased organic matter is linked to higher biological oxygen demand (Chiam and Sarbatly, 2011), gives more substrate for microbial degradation which leads to increased CO_2 production and consequently affects pH, and affects nitrification efficiency (Zhu and Chen, 2001). Interestingly the same response is seen in both systems, despite higher removal rate of particles in mRAS. As pH was actively adjusted to maintain appropriate levels, the similar pattern here is not surprising. One could assume there would be a difference in how much buffer was required for these adjustments, but no significant difference in mean added $NaHCO_3$ during the experiment was detected (Table 5). In other words, the two systems required similar effort to maintain pH (Fig. 6B), as well as alkalinity (Fig. 6C), at selected levels. The causes of the observed decreases in nitrate could be more complex, as discussed next.

5.1.2 Effects on development of biofilter efficiency

The development of TAN and nitrite mostly follow the expected trends indicating maturation of the biofilters, with an early peak of TAN followed by a peak in nitrite, and decreasing levels of each after their respective peak (Lekang, 2013). The second peak in TAN observed by the end of P1 deviates from this, and indicates a period of reduced nitrification rate, likely due to the coinciding pH fluctuation, which negatively affects nitrification (Lekang, 2013). The nitrite peaks at potentially harmful concentrations, but the relatively high salinity at the same time likely prevented mass mortality (Noble et al., 2018). The nitrate results indicate a more random development. If no active measures are taken for removal of nitrate, it should accumulate over time. Lack of precision in the Hach instrument used for nitrate measurement could to some extent explain the results, otherwise it is a strong indication that denitrification took place. As previously mentioned, sedimentation of uneaten feed particles took place near the outlet of the fish tanks, and sedimentation was also observed in the sumps in the water treatment room (Fig. 1). This, as well as thick layers of biofilm occurring all over the systems, created anoxic environments where denitrification could occur. When cleaning the system after the experiment had ended, bubbles arose from the sedimentations, which strongly supports that denitrification had been occurring during the experiment. Anoxic environments also increases the risk of production of hydrogen sulphide (H_2S) (Wedemeyer, 1996), which is toxic to salmon (Kiemer et al., 1995). Anecdotal evidence link decreasing nitrate levels in RAS with the presence of

H_2S, supported by knowledge from wastewater treatment where nitrate has been used to decrease hydrogen sulphide levels (Garcia de Lomas et al., 2006).

Low nitrate levels could also indicate incomplete nitrification and issues with biofilter performance, but the relative absence of increases in TAN and nitrite contradict this explanation. There are only indications of a slight increase in TAN and nitrite towards the end of P2, a period with rapid decrease in nitrate, and in TAN towards the end of P4. These are indications of reduced biofilter efficiency, which could have been caused by the increase in organic carbon (Zhu and Chen, 2001) or the reduction in pH (Lekang, 2013). The reduction in pH could in turn be a result of increased decomposition of organic matter by bacteria due to the increase in particulate organic matter, and correlates with the coinciding increase in total amount of bacteria reported by Nesje (2018).

5.1.3 Effects on fish performance

The periods of high load on the system (P2 and P4) appear to correlate with the periods of decrease in condition factor. Condition factor is known to decrease during starvation (Einen et al., 1998, Mørkøre et al., 2008), and it is possible that the high turbid water in this experiment decreased the appetite of the fish enough to affect condition factor. For the first high load period (P2), the effect is of comparable magnitude in both systems, while mRAS exhibits a stronger reduction in P4. As the fish in cRAS were exposed to higher turbidity than the fish in mRAS, and for a longer duration, it is possible that those fish acclimated to the effects of a high-turbid water to a higher degree than the fish in mRAS during the experimental period before P4. This could indicate that it was the rapid increase in particulate load on the system, rather than the baseline turbidity, that yielded a negative effect on condition factor. If that is the case, a constant turbidity might be preferable to fluctuating turbidity, even if the constant turbidity is higher than the fluctuating.

On the other hand, the change in condition factor might not be a direct effect of change in turbidity, but rather a consequence of the secondary effects associated with the accumulation of particles, as discussed above, or other correlating events. Several other events of deviation from optimal water quality occurs within P2, including a rapid reduction in water temperature (Fig. 4A) and high supersaturation of oxygen (Fig. 4B). Another important process which is

associated with a reduction in condition factor is smoltification (Farmer et al., 1978), which is discussed further in Chapter 5.2.2.

Whether turbidity affected any of the morphological welfare parameters, is difficult to determine. The only morphological welfare indicator that consistently scored lower than the maximum in both systems, was fin damage (Fig. 8C). Fin damage was also the only morphological structure to have significantly different probability of occurrence when looking at scores from all fish from the experiment (Table 8), being higher in cRAS. This could be linked to the difference in particles in the water, as fin erosion is supposedly associated with high total suspended solids (Wedemeyer, 1996). Although, as pointed out by Branson (2008), scientific evidence for this is lacking. Good et al. (2009) found significantly greater fin erosion when investigating rainbow trout (*Oncorhynchus mykiss*) in a RAS with very low water exchange rate, compared to a RAS with relatively high exchange. In the RAS with low exchange where fish yielded poorer fin condition, there was also significantly higher TSS, indicating a possible link between suspended solids and fin erosion. In the current study, the systems did not diverge in frequency of fin damage during the experiment (Fig. 8C), and significant difference was observed only when including the final sampling (Table 8). It is possible that fin damage frequency escalated during P4, in which case particles in the high turbid water and associated effects on water quality is a plausible explanation.

The lower fin scores were to a large extent due to observed fin erosion on the dorsal and pectoral fins, and especially the pectoral fin on the side of the fish positioned towards the centre of the tank when swimming against the current. In addition, it should be pointed out that it was frequently observed that in both systems, the fish appeared to prefer the corner of the tank containing the water inlet. This could indicate an area with slightly better water quality, or it could be because the inlet pipe constituted the only hiding spot in the tank. Regardless of cause, this suggests an area the fish might have been competing for. If the spot furthest from the centre of the tank was the most preferable, it could explain both the presence of fin damage, and which fins were affected, as the fins faced towards the centre would be most frequently targeted by fish competing to get to the corner. Fin damage is reported to heal rapidly in the temperatures experienced by the fish in both systems (Wedemeyer, 1996), which is a likely explanation of why the observed fin damages were rarely severe.

An interesting result in the present study, is the lack of gill damage. The increase in gill problems with the presence of particles in water is well established (Chapman et al., 1987, Bullock et al., 1994). Gills should be among the first morphological structures to be affected due to their delicate nature, but that was not observed in the samplings during the experiment. It is unlikely that the gills were completely unaffected, particularly during P4 with severely turbid water (Fig. 3C), but it is possible that the lesions were too small to be detected with the naked eye. The morphological inspection at the end of the experiment did not cover gills due to limited time, but in hindsight, this was a structure which should have been prioritised.

5.2 Temperature development, and associated effects

In this study, mean temperatures in mRAS and cRAS were significantly different. Given that the differences in temperature escalated when systems were fully separated (onset of P2), and differences were reduced when additional cooling of the high temperature water in mRAS was applied (week 7), it is safe to assume that a main driver behind the temperature differences (Fig. 4A, Table 5) was heat production in mRAS. This production was likely the result of increased pumping and friction associated with the operation of the membrane filter. Due to the design of the recirculation treatment loop, with S1, S2, biofilters and S3 from each system being located next to each other (Fig. 1), heat exchanges between the systems made temperatures in both systems correlate and made system specific temperature adjustments difficult. In addition, it is worth pointing out that the main method for temperature regulation was by room temperature adjustments in the room with the fish tanks, meaning that the water cooled down when it was in the fish room, but heated up during recirculation treatment, before returning to the fish (Fig. 1). Also, the degree of recirculation affected temperature, as the intake water had a lower temperature than the system water. With less recirculation (P3, Fig. 3A) the temperature of the system water decreased and stabilised (P3, Fig 4A).

5.2.1 Effects on growth

In this study, mRAS yielded higher growth rates than cRAS. Growth rates are known to increase with higher temperatures (Austreng et al., 1987), and the thermal growth coefficient (TGC) attempts to express growth independent of the temperature (Thorarensen and Farrell, 2011). Only SGR was significantly different, and not TGC (Table 6), which suggests that the difference in temperature between systems (with the average temperature in mRAS being 1.23 °C higher) is likely to be the primary factor causing the observed higher growth and

consequently higher end weight in mRAS compared to cRAS (Table 6). It is difficult to determine to which extent other factors might have influenced growth, but a significant difference in TGC during P4 (Table 7) indicates that temperature alone cannot explain the observed growth difference in this period. On the other hand, this difference is very small, and the TGCs displaying growth rates for the entire experiment were not significantly different. It is also important to consider that all calculated TGCs in this study might suffer from inaccuracy, as several of the assumptions that has to be met for TGC to be precise has been violated in this experiment (Jobling, 2003). Most prominently that temperatures in both systems were not constant, and were close to or over the upper limit of the temperature range of 4-14 °C where TGC is a good predictor of growth (Thorarensen and Farrell, 2011).

Observed growth rates in both systems (SGR and TGC, Table 6) are also lower than the potential growth rates at the respective temperatures in the systems (Austreng et al., 1987, Thorarensen and Farrell, 2011). This suggests suboptimal conditions for growth in both systems, for at least parts of the experimental period. The low growth rates can partially be explained by a period of growth depression in the weeks after stocking. Growth was calculated from time of stocking (week -2 of the experiment), and not from the start of the experiment (week 0), because of a decrease in measured mean weight compared to when the fish arrived (Fig. 7A). This decrease was determined to be too severe to accurately represent the population mean, even though some weight reduction could be expected following the stress associated with transportation, being put into water of higher temperatures than the fish were used to, and a short starvation period. An explanation of this likely measuring error could be that the fish who were most affected by the circumstances, were the ones who were sampled as they were unable to escape the dip net used for sampling. An important reason for low growth rates, however, is likely connected to the light regime (8L:16D), as short day lengths are known to yield lower growth rates than longer day lengths in juvenile salmon (Lundqvist, 1980, Saunders et al., 1985, Sigholt et al., 1995). In the current experiment, the 8L:16D regime was maintained to try to prevent smoltification.

5.2.2 Smoltification

It was determined to operate at light conditions of 8L:16D because the fish was already on a similar rearing regime when they arrived for this experiment, in order to try to prevent smoltification. The increase in photoperiod from winter to spring is an important cue for

smoltification (Bjornsson et al., 1989, McCormick et al., 1987), and not providing this increase (such as by rearing at constant 24L:0D) will inhibit parr-smolt transformation (McCormick et al., 1987). While rearing at constant 8L:16D will not provide the increase in photoperiod associated with onset of smoltification, other factors in this experiment (such as body size and relatively high temperatures in the water) is likely to have triggered the morphological and physiological transformations.

The observed changes in smolt index score indicates that onset of smoltification occurred relatively early in the experiment (Fig. 8B). It should be taken into consideration that changes in colouration (silvering, fin margins blackening) can occur in juveniles that are not functionally smolts (Wedemeyer, 1996). However, the increase in smolt index (Fig. 8B) combined with body size (Fig. 7A), periods of decrease in condition factor (Fig. 7C) and the capability of osmoregulating in seawater (Table 10) all suggests that smoltification to a large extent had occurred by the end of the experiment, even though smoltification was not intentionally initiated.

5.3 Oxygen, carbon dioxide, and associated effects

Apart from turbidity and temperature, the only other water quality parameters with significant different means between cRAS and mRAS, were oxygen and carbon dioxide. The results suggest mRAS had lower levels of oxygen and higher levels of CO_2 than cRAS. In contrast to turbidity and temperature, these differences are not easily explained by the membrane filtration. However, the difference in biomass and temperature both plays a role in explaining the observed difference in O_2 and CO_2.

5.3.1 Oxygen

With a higher biomass and temperature follows increased metabolic rate reflected in a higher consumption of O_2 (Andrew and M., 1999, Brett and Glass, 1973, Fivelstad and Smith, 1991), which corresponds with the development of a higher biomass in mRAS (Fig. 7). Interestingly, the O_2 levels starts to diverge with the onset of P2, indicating the oxygen consumption in the two systems might have been of a similar level until that point, and higher in mRAS from that point onwards. However, the explanation for the difference might be more complex. Coinciding with the diverging oxygen levels is the rapid increase in temperature in mRAS (Fig. 4A), which would have reduced the solubility of oxygen in mRAS. It is therefore possible that the water

entering the fish tanks contained different amounts of oxygen in the two systems. As dissolved oxygen was only measured in the outlet of the fish tanks, and the actual total biomass in the tanks were not known during the experiment, there is no way to know the exact amount of consumed oxygen. In addition, it should be noted that there were several occasions where the mechanisms for emergency oxygenation of the water malfunctioned, causing excess oxygenation and up to 200% saturation (Fig. 4B) for periods of less than 24 hours, which has affected the calculated average O_2-levels.

When it comes to whether the fish in either system suffered any effects from the oxygen levels, it is at least unlikely that they were exposed to too low concentrations. Low oxygen concentrations reduce growth (Thorarensen and Farrell, 2011), which is the primary risk in aquaculture, which is why guidelines focus on lower limits (Noble et al., 2018). However, hyperoxic environments will also affect the fish, lowering ventilatory frequencies (Dejours et al., 1977), affecting blood acid-base regulation (Wood and Jackson, 1980), impairing gill function through oxidative stress (Brauner et al., 2000), and affecting behaviour and possibly reducing growth (Espmark and Baeverfjord, 2009). In addition, supersaturation of oxygen could cause gas bubble disease (Espmark et al., 2010). Gas bubble trauma can occur at high total dissolved gas supersaturations (Wedemeyer, 1996). A strong indication of gas bubble disease is bulging eyes, which was observed in both systems, and amounted for the most severe damages out of all measured morphological welfare indicators (fins, opercula, gills, snout, mouth and eyes). The measured oxygen levels in this study indicates the fish were exposed an overall supersaturation of oxygen (average saturation of 97% in mRAS and 103% in the outlet of the fish tanks, meaning higher concentrations in the inlet), in addition to shorter periods of severe supersaturation (Fig. 4B). In addition to supersaturation of oxygen, the observed sporadic nitrate levels indicate denitrification which produces nitrogen gas, and supersaturation of nitrogen is much more likely to cause gas bubble disease than oxygen (Summerfelt et al., 2001). It should be noted that although a significantly higher frequency of occurrence was detected in mRAS among the fish sampled at end of the experiment, this difference disappeared when looking at all results from the entire study period (Table 8).

5.3.2 Carbon dioxide

The relatively high oxygen levels, in combination with biomass and temperature in the systems, could all be linked to the significant difference in carbon dioxide. CO_2 is produced in a RAS

by excretion of the fish, as well as by heterotrophic bacteria. Excretion of CO_2 by Atlantic salmon increases with increasing oxygen consumption (Espmark and Baeverfjord, 2009) and feed ration (Forsberg, 1997). As discussed above, there are indications of a higher oxygen consumption in mRAS, and while both systems received an equal amount of feed, the feed conversion rate was lower in mRAS than in cRAS (FCR = 2.58 and 3.27, respectively). The FCR values suggests both systems were severely overfed, when compared to the average FCR of 1.15 for Atlantic salmon produced in Norway in 2013 (Ytrestøyl et al., 2015). Furthermore, the difference in FCR indicates a higher consumption of the available feed in mRAS than in cRAS. While the CO_2 produced by bacteria in the systems is more difficult to assess, it could be assumed that in total, the CO_2-production was higher in mRAS than in cRAS, which would explain the significant different means. It should be noted that the means and the difference between them is small (1.55mg/L in mRAS, 1.46mg/L in cRAS), and thus it could be argued that the measured levels of CO_2 are too low and regular to be accurate (Fig. 6A). Measuring equipment malfunction is a possible explanation, but as the measuring device was controlled multiple times, that is unlikely. It should be noted that the OxyGuard device used in this experiment only detects the free dissolved CO_2 gas, and an alternative explanation for the low values is that the combination of a relatively high pH (>7.5 in both systems for most of the experiment), an alkalinity of ≈50 mg/L (as $CaCO_3$) and the temperature kept most of the CO_2 as HCO_3^- (Summerfelt, 1996, Wedemeyer, 1996).

There is conflicting evidence regarding which concentrations of CO_2 that causes detrimental effects, Wedemeyer (1996) claims the levels which negatively affect salmonids starts at >20 mg/L, with respiratory distress occurring at ambient concentrations >40 mg/L, and death occurring >100ppm. Potential long term effects of sub-lethal concentrations include nephrocalcinosis (Fivelstad et al., 2003), and results from Khan et al. (2018) suggests that chronic exposure to any concentration (down to 2.9 mg/L) might have negative effects on production performance. On the other hand, a recent study by Good et al. (2018) found that post-smolt reared in high alkalinity freshwater at 20mg/L and 8mg/L CO_2 in RAS performed equally. Considering this, it is highly unlikely that the fish in this study suffered any major effects of the low CO_2-concentrations measured in this study.

5.4 Other developmental trends worth noting

5.4.1 Response to handling stress

The observed changes in the plasma glucose response in week 13 and 18 compared to week -1 (Fig. 9, top), could be correlated to smoltification. Carey and McCormick (1998) found that smolts require longer time to recover back to baseline values, compared to parr. As indications of smoltification were present before week 13 and 18 (high smolt index scores, Fig. 8B), it can be assumed that the elevated glucose levels 6 hours after handling stress is linked to the fish having mostly transitioned into smolts. Both the plasma glucose and lactate levels were arguably high already before handling stress (Fig. 9) at all weeks, compared to pre-stress measurements from other studies (Noble et al., 2018). This suggests the fish in both systems might have been slightly stressed even before being intentionally exposed to a stressor, although the cause is difficult to determine.

The response in lactate to handling stress indicates a much higher variance within the mRAS fish than within cRAS (Fig. 9, bottom). An interpretation is that some fish in mRAS did not exhibit a response requiring production of plasma lactate, but those that did responded more severely than fish from cRAS. This could indicate a higher initial handling stress tolerance in mRAS, but with a stronger response once a threshold was reached.

5.4.2 Mortality

Overall, the mortality was low with only 3 fish dying in each system during the experiment. Interestingly, 5 out of the total 6 fish died early on, indicating that a mortality inducing event occurred in both systems. No single parameter is a good explanation of the observed mortality event, especially since the total mortality was so low. It can be assumed that those who died were fish struggling the most in the systems, and the combination of peak nitrite levels (Fig. 5B), high temperatures (Fig. 4A) and possibly other stressors proved too much for those individuals.

5.4.3 Other examined morphological welfare indicators

Overall, observed lesions for most of the measured morphological welfare indicators (mouth, snout, opercula, gills) were considered minor and had a relatively low frequency of occurrence for the total populations, the exceptions are the previously discussed fin damages (mostly non-severe, but relatively high occurrence) and eye damages (severe, but low occurrence). The

analysis of overall severity of damages sustained during the experiment, indicate significantly different frequency of the group containing scores of 3 or less, with higher occurrence in cRAS compared to mRAS (Table 9). This suggests a higher probability of sustaining slightly more severe damages in cRAS than in mRAS, although the effect disappears when including less severe damages (scores ≤ 3.5) or excluding score of 3 (scores ≤ 2.5). In other words, sustained damages were mostly minor in both systems, but slightly more severe in cRAS.

Shortened opercula were observed in both systems, but not to a large extent (< 4% of the total population in both systems, Table 8), and mostly non-severe cases. In aquaculture, a presence of deformed opercula is common, and while the exact causes are uncertain, it might be related to rearing conditions (Noble et al., 2018). Opercula damage are also reported to be less common in RAS than in flow-through systems (Kolarevic et al., 2014). Vertebral deformities were not actively looked for during the experiment, but severe cases were observed in two fish from mRAS at the end of the experiment. The exact mechanisms causing development of skeletal deformities is not known, but it is likely the result of an interaction between multiple factors, or it could be due to natural variation, which is a reasonable explanation in this case given the low frequency of occurrence (Noble et al., 2018).

At the end of the experiment, damage to skin on top of the had was also seen for the first time. These damages, as well as some of the other damages observed on the final sampling day, is likely to be the result of the sampling procedure. After being euthanized, fish from each tank was collected and placed in separate buckets. Absence of gentle handling of the fish in this procedure is a reasonable explanation of some of the damage to head and snout, and could possibly to some extent have affected fins. Especially the observed damages to the head region could be explained by rough handling, which is supported by the fact that this kind of damages were not observed in any previous sampling.

When it comes to the internal morphology, no obvious differences were observed between systems. Overall, these procedures suffered from being hastily performed, as they were performed between blood samplings and time was of the essence to secure good blood samples. A more thorough investigation might have revealed more, such as development of gonads.

5.4.4 Salinity

Both systems experienced similar changes in salinity throughout the experiment, only diverging slightly in P3 (Fig. 4C). All changes in concentration were due to manual adjustments, with initial salinity being higher than the aimed concentration of 1-2 ppt due to technical imprecision. Manual adjustments were performed to try to lower the salinity in both systems, which resulted in a slow decrease due to high retention time in the system as a consequence of high daily recirculation.

5.4.5 Other potential stressors

Substantial amounts of adjustments to water treatment components, and attempts to unclog pipes in the final weeks of P2, is likely to have contributed to the total level of experienced stress in both mRAS and cRAS, although of comparable magnitude. Daily procedures involving measurements of water quality in the outlet of the fish tanks, and particularly opening of the lid to investigate mortality, can also have affected the total stress experienced by the fish. Even though these procedures were the same for both systems, the fish might have been affected differently. A distinct difference in behavioural response of the fish to the opening of fish tank lids was observed during the end of P4, with no response in the high turbid cRAS, but substantial escape-associated behaviour in mRAS. Although this effect of turbidity on experienced stress was not quantifiable with the current setup, it seems reasonable that turbid water might also have a positive effect on stress by reducing the impact of visual stressors.

6 Conclusions and further recommendations

The results from the study suggests that temperature, turbidity, oxygen saturation and CO_2 are the water quality parameters with the highest discrepancies between the two systems. The membrane filtration is assumed to be the primary cause of observed differences in temperature and turbidity, while oxygen and CO_2 were more likely caused by differences in biomass and temperatures in the systems. Several of the other measured water quality parameters displayed changes in response to increased particulate load, but developed in a similar pattern in both systems.

Discrepancies were also observed in fish performance. Specific growth rates (SGR) for the entire experimental period were higher in mRAS compared to cRAS, but no difference was observed in the thermal growth coefficient (TGC). Therefore, temperature is assumed to be the primary cause of the difference in growth rates, although results from the last period with high particular load suggests that turbidity and associated effects might also have been involved. Condition factor was higher in fish from mRAS during most of the experiment, but fish from cRAS yielded slightly higher and significantly different values at the end of the experiment. Analysis of morphological welfare indicators implies there was a higher probability of sustaining fin damages in cRAS. Severe eye damages were observed in both systems, appearing more frequent in mRAS at the end of the experiment, but the membrane filtration is unlikely to be the direct cause. Results imply a higher probability of sustaining non-severe damages in cRAS, with an overall low frequency of severe injuries in both systems. In total, fish performance in the two systems were at comparable levels, apart from the difference in growth.

Overall, the results imply that under the present conditions, the presence of particles have limited direct effects on performance of Atlantic salmon. However, particles are observed to be involved in several negative effects on other water quality parameters, and increasing the risk of uncontrolled anoxic environments. For Atlantic salmon farming, membrane filtration might prove more beneficial for other life stages than the studied parr/smolt, such as the more sensitive early life stages or in high density post-smolt production. If the effect on temperature is taken into account and potentially utilised, a membrane could be a good addition to improve removal of the smallest particles and increase stability in turbidity.

Future studies utilising membrane filtration to study the effects on water quality and fish performance are recommended to perform multiple experiments of shorter duration, instead of one long experiment with varying operating conditions. Also, because particles are involved in complex interactions in RAS, the optimal levels of TSS and turbidity might be difficult to assess. Based on the results from this study, it is suggested to investigate whether a limit to change over time, including duration and magnitude of increase, might be more expedient for developing appropriate guidelines. In addition, other compounds that might accumulate with increased intensification should be further investigated, preferably in addition to particles, in order to estimate the relative risk of negative effects from each of the accumulating compounds. If enough knowledge can be assessed to determine appropriate levels of, and the interaction between, particles, turbidity and fish performance in RAS, optimisation is likely to increase production stability.

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8 Appendix I

Total number of fish (n) in each fish tank at stocking, with total weight of fish per tank ($\mathbf{W_{0\text{-}Tank}}$), biomass density (kg/m^3) and mean weight of fish per tank ($\overline{\boldsymbol{W_{o-Tank}}}$) (Table 11).

Table 11 Overview of tanks in each system, mean wet weight of fish ($\overline{\boldsymbol{W_{o-Tank}}}$), total wet weight of fish **($\mathbf{W_{0\text{-}Tank}}$),** density and number of fish (n) in each tank after fish had arrived.

System	Tank	$\overline{\boldsymbol{W_{o-Tank}}}$ (g)	$\mathbf{W_{0\text{-}Tank}}$ (kg)	Density (kg/m^3)	n
cRAS	1	46.13	2.768	6.92	60
cRAS	2	46.70	2.802	7.01	60
cRAS	3	45.02	2.836	7.09	63
cRAS	4	49.90	2.894	7.24	58
cRAS	5	41.05	2.504	6.26	61
cRAS	6	41.48	2.489	6.22	60
mRAS	7	45.45	3.136	7.84	69
mRAS	8	46.73	2.897	7.24	62
mRAS	9	45.17	2.71	6.78	60
mRAS	10	45.02	2.701	6.75	60
mRAS	11	48.63	2.869	7.17	59
mRAS	12	47.59	2.903	7.26	61

9 Appendix II

Overview of sampling dates/week of experiment for fish performance sampling, with sample size and what was sampled for (Table 12). Not all data from the samplings were used in the final results. At the final day, the remaining fish were sampled in two samplings. First 24 from each system, using the scoring index from Table 3 and taking blood samples. Secondly, the remaining fish was slaughtered, measured and given welfare scores based on the simplified scoring index.

Table 12 Sample dates/weeks, sample size and what was sampled for, for all fish performance samplings.

Date, week of experiment	Number sampled	Sampled for
24.01.2017, week -2	Total weight per tank	Weight
03.02.2017, week -1	23 (Start) 8 for 0h, 8 for 1h, 7 for 6h)	Morphology, Handling stress test
06.02.2017, week 0	12 (mRAS), 12 (cRAS), 2 per tank	Morphology, Blood (Baseline)
28.02.2017, week 3	24 (mRAS), 24 (cRAS), 4 per tank	Morphology, Blood (Baseline)
03.04.2017, week 8	25 (mRAS), 26 (cRAS) (+1 and +2 fish extra due to netting error) ≈ 4 per tank	Morphology, Blood (Baseline)
20.04.2017, week 10	24 (mRAS), 24 (cRAS), 4 per tank	Morphology, Blood (Baseline)
11.05.2017, week 13	18 (mRAS), 18 (cRAS) 3 per tank (1 for each treatment, 0h, 1h, 6h)	Morphology, Handling stress test
22.05.2017, week 15	24 (mRAS), 24 (cRAS), 4 per tank	Blood (Baseline), (Morphology)
12.06.2017, week 18	18 (mRAS), 18 (cRAS) 3 per tank (1 for each treatment, 0h, 1h, 6h)	Morphology, Handling stress test
13.06.2017, week 18	24 (mRAS), 24 (cRAS), 4 per tank	Morphology, Blood (Baseline)
13.06.2017, week 18	6 (mRAS), 6 (cRAS), 1 per tank	Seawater tolerance test
13.06.2017, week 18	193 (mRAS), 183 (cRAS), the rest of the fish still in the tanks	Morphology (simplified), End of experiment