A comparison of two seawater adaptation strategies for Atlantic salmon post-smolt (Salmo salar) grown in recirculating aquaculture systems (RAS): Nitrification, water and gut microbiota, and performance of fish

Ragnhild O. Fossmark⁎, Kari J.K. Attramadal, Kristian Nordøy, Stein W. Østerhus, Olav Vadstein

Department of Civil and Environmental Engineering, NTNU – Norwegian University of Science and Technology, N-7031 Trondheim, Norway
Department of Biotechnology and Food Science, NTNU – Norwegian University of Science and Technology, N-7491 Trondheim, Norway
LetSea AS, N-8801 Sandnessjøen, Norway

ARTICLE INFO

Keywords:
Recirculating aquaculture system (RAS)
Salt acclimatization
Nitrification
Atlantic salmon post-smolt
Saline RAS
Microbial community composition

ABSTRACT

The land-based production phase in Norwegian Atlantic salmon farming has the past years been extended to include post-smolt for an increasing number of farmers. Post-smolt production can involve introduction of brackish/seawater to a recirculating aquaculture system (RAS) that is acclimatized to freshwater for the earlier stages of salmon production. A change from freshwater to seawater shifts the environmental conditions for fish, bacteria and water treatment processes in RAS. Two regimes for salinity increase were studied to evaluate the effects on nitrification functionality, water and gut microbiota and fish performance on land and in the sea cages. A fish group of 200,000 salmon parr were stocked in a brackish water RAS (bRAS) at 3‰ salinity. After the fish had smoltified the group was split in two, one group was kept in bRAS and the other was moved to a RAS operated at 28‰ salinity (sRAS). The bRAS was operated with a gradual increase in salinity from 3 to 26‰ over a period of 28 days, whereafter both groups were moved to two separate sea cages. Bacterial communities of water, biofilter biofilm and fish faeces were characterized by 16S rRNA amplicon sequencing. Nitrification capacity tests at different salinities were performed on biofilter media from bRAS, to evaluate short term robustness to salinity changes. Ordination based on Bray-Curtis similarities showed that in water samples in bRAS, the bacterial communities were stable from 12 to 26‰ salinity increase. The faecal microbiota of the fish showed high inter-individual variation within fish tanks, suggesting stochastic processes/drift to affect the community structures in addition to salinity increase. The same nitrifying bacteria were present in bRAS (throughout the salinity increase) and in sRAS, showing that these nitrifiers could adapt to salinities from 3 to 26‰, and 28‰. After the sea cage phase, fish from the sRAS system had in total 2.9% higher weight than the fish from bRAS, however the mortality was 15% higher in the sRAS group. Salinity was a driver for succession in RAS, and other factors such as organic load in the water and stochastic processes in the host also affected the bacterial community dynamics.

1. Introduction

Production of Atlantic salmon (Salmo salar) post-smolt, or large smolt, on land in recirculating aquaculture systems (RAS) is increasing in Norway. In traditional salmon farming, the fish are produced in land-based systems with freshwater or brackish water with low salinity (~0–3‰) from hatching to smoltification. Then they are moved to net pens in the sea for grow-out to market size. Handling stress during the transfer to sea, and exposure to salmon lice and delicing treatments, can reduce growth and increase mortality of the fish in the sea cages (Iversen et al., 2005; Nilsen et al., 2017). Salmon lice have become a significant challenge for salmon farmers (Abolofia et al., 2017), and is one of the drivers for extending the land-based phase to produce post-smolts to reduce the time the fish are exposed to lice in the sea cages (Dalsgaard et al., 2013). Post-smolt production will increase the fish size before transfer to sea, which is hypothesized to make the fish more robust for the exposed conditions at an open sea cage and possible sea lice attack. A second driver for increased production time on land is higher utilization of the Maximum Allowed Biomass (MAB) at the sea locality given in the concessions for the fish farm in Norway (Lekang
et al., 2016).

From an operational perspective, the question of whether the post-smolts should be reared in freshwater or brackish/saltwater in RAS is discussed. Atlantic salmon have been grown to market size in RAS with only freshwater (Davidson et al., 2016), however problems with early sexual maturation was shown to be a production barrier as it reduces growth and flesh quality (McClure et al., 2007). Furthermore, the natural anadromous life cycle of the salmon is overlooked, and the fish may use energy to change the osmoregulation by smolting and desmoltifying, which will also negatively affect the growth (McCormick and Saunders, 1987; Jørgensen and Jobling, 1994; Johansson et al., 2016). It has been shown that salinity over 15% in Atlantic salmon cultivation can inhibit desmoltification (Mortensen and Damsø, 1998), indicating that optimal growth of the post-smolts should be above this salinity. Higher salinity can be introduced by moving the smoltified fish from a freshwater system to a post-smolt system run with brackish or seawater (as done by e.g. Erko Settefisk AS, Stord, Norway). This may lead to stress for the fish due to handling and transport. Alternatively, seawater can be introduced into the RAS used for freshwater cultivation of juveniles. This results in a major change in the environmental conditions for the water treatment system and the functionality of the bacterial populations adapted to the freshwater system. It could, however, be a better option as it is a more gentle way of changing the environment for the fish, and because the transportation is reduced. Bacteria in RAS are vital for conversion of waste nutrients to maintain high water quality (Blancheton et al., 2013). Furthermore, bacteria have an important function for fish health, by facilitating nutrient adsorption and digestion in the gut, and for functional development including the immune system (Fraune and Bosch, 2010; Gomez et al., 2013; Llewellyn et al., 2014). However, little is known about the bacterial dynamics and the functionality of e.g. nitrifying bacteria during the transition from freshwater to seawater in RAS.

The biofilter function in RAS is especially vulnerable to salinity increase. Several studies have shown negative effects of salinity increase on nitrification efficiency and changes in bacterial community dynamics of ammonia oxidizing (AOB) and nitrite oxidizing bacteria (NOB) in wastewater treatment (WWT) (Uygur and Kargi, 2004; Aslan and Simsek, 2012; Wang et al., 2015; Cortés-Lorenzo et al., 2015). It is, however, not straightforward to transfer this knowledge to RAS for fish production as the studies have different; 1) environmental biofilm history, 2) species present in the biofilter, 3) temperatures and pH (Moussa et al., 2006), and 4) the concentrations of total ammonia nitrogen (TAN) and nitrite nitrogen (NO₂⁻N) in RAS with Atlantic salmon are substantially lower than in WWT. The latter can affect the nitrifying community dynamics and nitrification efficiency, as TAN usually is the rate limiting substrate in RAS, compared to oxygen in WWT (Chen et al., 2006; Rusten et al., 2006). TAN and NO₂⁻N in RAS with Atlantic salmon production should be below 2 mg L⁻¹ and 0.1 mg L⁻¹, respectively, to avoid toxicity for the fish (reported for freshwater RAS from the Norwegian Food Safety Authority, 2016). Nitrification efficiency has shown to decrease after abrupt salinity changes as the nitrifying bacteria are inhibited by osmotic stress (Gonzalez-Silva et al., 2016; Kinyage et al., 2019). Increasing the salinity in a RAS can cause accumulation of toxic levels of TAN and nitrite, and potentially lead to mortality of the fish. Studies have shown that the nitrification process/efficiency can recover after or during increased salinity (Bassin et al., 2012; Quartaroli et al., 2017; Navada et al., 2019). Bacteria can adapt to higher salt stress (Zahran, 1997; Oren, 2011), and the increase of salinity in a RAS with fish is therefore possible.

RAS is a complex ecosystem with bacteria associated with water, fish and biofilter. Different salinities have been shown to alter the bacterial community structures in the water of RAS (Bakke et al., 2017; Rud et al., 2017), and the gut microbiota of salmon change during the transition from freshwater to seawater (Rud et al., 2018). It is not clear how much of the bacterial community dynamics that is a physiological salinity adaptation process relative to succession causing change in community structure and introduction of new species. More knowledge is needed to understand the bacterial adaptation versus succession during such shifts in environmental conditions in RAS. The fish are exposed to a sudden and major change in environmental microbes during the transfer to sea at a vulnerable stage. Bacterial diseases documented in sea cages have been associated with the transfer of smolt to the sea (Egge et al., 1997; Johansson et al., 2016; Hjeltnes et al., 2019). It is not known whether the bacteria associated with the smolt on land prior to transfer to sea affects the susceptibility for diseases at sea. How the changes in salinity affects the microbial water quality and nitrification efficiency in RAS, and linking it to fish performance before and after transfer to sea have not been addressed before, and was the motivation for our study.

We hypothesize that a gradual increase of salinity in RAS aclimatize both the microbiota associated with the fish and the biofilter, resulting in more robust fish at transfer to sea and a more functional biofilter on land. This hypothesis was tested experimentally in two regimes for brackish to seawater transition in production of Atlantic salmon post-smolt in RAS with subsequent transfer to sea. One regime was a gradual increase in salinity in RAS, whereas the other was a direct transfer of smolt to a RAS with high salinity. More specifically we evaluated 1) how a gradual increase in salinity in RAS affected the microbial community structure and dynamics in water and biofilter, 2) how the nitrification efficiency of the biofilter adapted to the two regimes for change in salinity, and 3) how the different salinity regimes affected fish growth and gut microbiota before and after transfer to sea cages.

2. Materials and methods

2.1. Experimental set-up and operational conditions during the study

The experiment was carried out at the LetSea RAS facility Bjørn and sea cage locality Havstein, both on/ by Donna (Nordland, Norway, 66°05’ N 12°31’ E). Two separate commercial scale RAS were used for the study. One system was a seawater/high salinity brackish water RAS (sRAS) that had previously been operated with 18–33% salinity for 3 years. The other system was identical, but a brackish water RAS (bRAS) operated with 3–5% salinity for 6 months before this experiment. Prior to that, bRAS had been operated with 20% salinity for 3 years. Seawater was pumped into the facility from 140 m depth, treated with a mechanical screen (200 μm) and UV. The freshwater came from the municipal freshwater distribution system. For both systems, seawater and freshwater were mixed to give their respective salinities. Each system (Fig. 1A), delivered by AKVA Group, had a total volume of 1200 m³. They included 18 fish tanks (34 m³) each and a water treatment loop with particle removal through a mechanical drum screen sieve (mesh 60 μm), CO₂-degasser and a split loop with 1/3 of the workflow going through a so-called fixed fluidized biofilter (147 m³, 50% filling, carriers with specific surface area of 600 m² m⁻³, giving a total biofilm area of 44,100 m²). Make-up water was 1% of total system volume per hour (12 m³ h⁻¹), giving 75% water recirculation per day. The pH was controlled with automatic addition of 0.3 M sodium hydroxide (NaOH).

The fish were fed commercial pellets from automatic feeders (on land: Intro Q 200, at sea: Energy X 1000 and Power Extreme 2500, Biomar).

Atlantic salmon (Salmo salar) parr with average weight of 37 ± 11 g (± SD), were stocked (200,000 individuals) in bRAS (06.03.2018). When the fish had smoltified and reached approximately 100 g (01.06.18), the smolt were randomized and split in two groups. One group (100,000 fish) remained in bRAS at 3%, whereas the other group (100,000 fish) was stocked in sRAS at 25% (Fig. 1B). The fish were counted with an automatic fish counter (AquaScan, Norway) during stocking, to ensure the same number of individuals in each system and fish tanks. The post-smolts were kept in the two experimental RAS for 60 days to acclimatize and to resume appetite after...
having been moved. The salinity in sRAS was increased from 25 to 28‰ after the 60 days, and remained stable through the rest of the experiment. In bRAS the salinity was slowly increased over a period of 28 days (03.08.2018–31.08.2018). The salinity was increased with 0.5 to 1.0‰ per day. Seawater was continuously mixed into the buffer tank in bRAS, and the salinity was usually 1–2‰ higher in here than in the fish tanks as it took some time for the water to mix in the system. The experimental plan was to increase the salinity in bRAS as much as possible without exceeding a concentration of 2 mg L⁻¹ TAN and 0.5 mg L⁻¹ NO₂-N (Norwegian Food Safety Authority, 2016) in the 28 days. These concentrations were measured daily, and if the concentrations reached the upper threshold, the feeding in bRAS was stopped and the seawater flow into the buffer tank was reduced. After the 60 days, and NO₂-N concentrations declined, feeding was resumed. At day 28, a salinity of 26‰ was achieved. After the termination of the salinity experiment, both fish groups were transported separately in a well boat to two individual sea cages at Havstein. The fish grew in the sea cages for around 1 year to a size range of 4.6–4.8 kg.

2.2. Daily management, measurements of water quality and fish weighing

All fish tanks/sea cages were inspected daily, and dead fish were removed and registered. In both RAS, water quality variables were measured in the water of the level tank (Fig. 1A). Temperature and oxygen were logged automatically every 10 min in the fish tanks. Salinity was measured with a handheld salinity meter (OxyGuard, Denmark). Total ammonia nitrogen (TAN), nitrite and pH were analysed with a Photometer 7100 (Palintest, UK). Dissolved CO₂ was measured with a CO₂-analyser (OxyGuard, Denmark) once a week. All other water quality variables were measured daily. The biofilter was backwashed two times a week. At each weighing of the fish on land, 25 individual fish from each tank were weighed manually (sum: 450 fish per system) and put back into the fish tanks. After the sea cage phase, all the fish were transported to the MOWI salmon slaughterhouse on Herøy (Nordland, Norway) for weighing and slaughtering.

2.3. Sampling for microbiological analysis from water, biofilter biofilm and salmon faeces

Samples for microbiological analysis were collected in both sRAS and bRAS when the salinity of bRAS was 3, 6, 12, 18 and 26‰. Samples of the water were collected also after the fish groups were split. Water samples were collected in the outlet of three fish tanks and in water going in and out of the biofilter. The water samples (approximately 200 mL) was filtered with 60 mL syringes through sterile 0.22 μm filters (Sterivex™) to collect bacteria. To collect biofilm carriers form the biofilter, metal grids which held the carriers together had to be drilled open. The middle of the biofilter was most accessible for this operation, and two biofilm carriers were collected from this location. Fish for faecal sampling were netted from the fish tanks into a smaller tank and euthanised within the guidelines for animal welfare given by the Norwegian Food Safety Authority (with an overdose of anaesthetics, Benzooak vet. 200 g L⁻¹). At each sampling, four fish were taken from the same three fish tanks as the water samples were collected. The faeces were squeezed out of the gut of the fish into a petri dish, and afterwards transferred to a sterile 2 mL Cryotube. From the sea cages, faecal samples were collected from 10 fish in each cage 3 weeks after sea transfer. All samples were stored at −20 °C until further analysis.

2.4. Nitrification capacity stress test

Nitrification capacity stress tests were performed to determine the maximum ammonia oxidation capacity (AORmax). This was to evaluate short term robustness to salinity changes of the carriers in bRAS when the salinity of the system was 5, 12 and 20‰. Freshwater and seawater mixed to salinities of 5, 12, 20 and 28‰, respectively, were added to four 1 L batch reactors. Biofilter carriers from bRAS were put in the reactors (30% filling, 220 carriers giving a total surface area of 0.18 m² in each reactor). The water volume was 0.95 L and the carrier volume was 0.30 L in each reactor. Ammonium sulphate ((NH₄)₂SO₄) was added to each reactor to feed the ammonia oxidizing bacteria (AOB). Start concentration of TAN was 15 mg L⁻¹ in the first stress test when tank salinity was 5‰, and 24 mg L⁻¹ for the tests when tank salinity was 12 and 20‰. The nitrite oxidizing bacteria (NOB) were only fed nitrite from the oxidized ammonium. The reactors had aeration from...
aquarium pumps to maintain oxygen concentration of approximately 8.2 mg L$^{-1}$ in each reactor (measured with a Handy Polaris 2 dissolved oxygen meter, OxyGuard, Denmark). The capacity tests were run for 210 min, with samplings for TAN and NO$_{2}$-N after 10 min and then every 30 or 60 min. Approximately 20 mL of water was sampled from each reactor with a syringe and filtered through a glass microfiber filter (Whatman GF/F, GE Healthcare, UK) for particle removal. TAN, NO$_{2}$-N and pH were measured in the filtrate with Photometer 7100 (Palintest, UK). Sodium bicarbonate (NaHCO$_{3}$) was added to the reactors to maintain the pH at 7.5–7.9, and the temperature was 14 ± 1 °C throughout the capacity tests.

2.5. Bacterial community composition

Bacterial community composition was determined by high throughput sequencing of PCR amplicons of a ~450 base pair long stretch of the 16S-rRNA gene. DNA extraction was conducted with the DNeasy 96 Blood and Tissue kit (Qiagen, Germany). The filters with water samples and biofilm carriers were cut into small pieces with a sterile scalpel and put into tubes supplied by the kit. Faecal samples were transferred directly to the tubes. The manufacturers’ protocol was followed with minor alterations. An extra lysis step was added in the beginning of the protocol to ensure lysis of Gram-positive bacteria. This was done with an enzymatic lysis buffer (180 μL) consisting of 2 mM EDTA, 20 mM Tris-HCl (pH 8), 1.2% Triton and lysozyme (20 mg/mL), and 1 h of incubation at 37 °C. PCR and Illumina sequencing was done according to Fossmark et al. (2020). The exception was that the V3 and V4 region of the 16S rRNA gene was targeted for sequencing, by the use of forward primer 338F 5′- tgc tgg gca gcc tca gat gtt tat aag aca cag nnnn CCT AGG GGW GCC AGC AG-3′, and reverse primer 805R 5′- gtc tgg gct ggg cgg aag tgt gta gaa gca g nnnn GAC TAC NVG GGT ATC TAA RCC-3′. Illumina adapter sequences are in lower case letters. The USEARCH pipeline (version 9.2) was used to process the Illumina sequencing data. All steps were done according to Fossmark et al. (2020), with the exception that reads shorter than 400 base pairs were considered co-amplified unwanted reads (e.g. mitochondrial salmon DNA), and were therefore filtered out. For water and faecal samples, the Ribosomal Database Project (RDP version 16; Cole et al., 2013) was used as reference to assign taxonomy to the OTUs. For the biofilter biofilm samples, the taxonomic assignment for nitrifying bacteria was rather poor or not classified at all with the RDP reference database. Given that nitrifying activity was observed in the experiment, this contradicted the classification given by RDP. For that reason the biofilter biofilm samples were checked with a different database, i.e. Microbial Database for Activated Sludge (MiDAS version 3: Nierychlo et al., 2019). Using MiDAS for taxonomy assignment, nitrifying OTUs were classified at high confidence threshold and the results reported for biofilter biofilm microbiota is therefore from MiDAS. The faecal samples contained many OTUs from the phylum Cyanobacteria, including chloroplasts. These OTUs were removed from the dataset before further analysis. To avoid bias due to variation in sequencing depth between samples, the water and biofilm samples were normalized to 20,000 reads (lowest read count), whereas the faecal samples were normalized to 4000 reads (lowest read count after removal of reads representing Cyanobacteria). OTUs of interest were further investigated with the SeqMatch tool for type strains at the RDP website and the BLAST search tool (Altschul et al., 1990).

2.6. Data analysis and statistics

The program package PAST version 3.21 (Hammer et al., 2001) was used to perform statistical analyses and to calculate bacterial community diversity index. Shapiro-Wilk tests (Shapiro and Wilk, 1965) were performed to check for normality in the water quality variables and fish weight data from the land phase. Two-Sample t-tests were used on data that were normally distributed, and Mann-Whitney-Wilcoxon-tests were used on data that did not. After the sea cage phase, the fish were automatically divided by size into weight-groups in the ranges 1–2, 2–3, 4–5, 5–6, 6–7 and over 7 kg. Afterwards all the individuals in each range were weighed, and total weight in each range was given. The average weight was based on the sum of all the fish slaughtered divided by total number of individuals. To estimate the number of individuals in each weight range, we assumed that the average weight for each of the size classes were 1.5, 2.5, 3.5, 4.5, 5.5, 6.5 and 7.5 kg. A Chi-square test ($\chi^2$) was performed on a contingency table consisting of the estimated numbers of fish in each range in bRAS and sRAS to determine if the size frequency was significantly different between bRAS and sRAS. The Alpha-diversity index of the bacterial communities was OTU richness (total number of OTUs). Beta-diversity was calculated based on the presence/absence-based Sørensen-Dice similarity and the abundance-based Bray-Curtis similarity (Chao et al., 2006). Ordination by Principal Coordinate Analysis (PCoA) with normalized and square root transformed data based on Bray-Curtis similarities was used to visualize the similarities and development (succession) of the bacterial communities in bRAS and sRAS. One-way permutational multivariate analysis of variance (PERMANOVA) with Bray-Curtis similarities was performed to test for statistically significant difference between different groups of samples (Anderson, 2017). Maximum ammonia oxidation rates (AOR$_{\text{max}}$) during the capacity stress tests were determined from linear regression of TAN concentration versus time in the reactors.

3. Results

3.1. Nitrification and water quality in bRAS and sRAS

During the salinity increase, the concentrations of TAN and NO$_{2}$-N in bRAS (Fig. 2) were thoroughly monitored to prevent toxic concentrations for the fish. As the salinity increased from 3‰ to 7‰, TAN and NO$_{2}$-N increased from 0.75 to 1.8 and 0.1 to 0.3 mg L$^{-1}$, respectively. Then the feeding was stopped, and the concentrations dropped to 0.4 mg L$^{-1}$ for TAN and 0.18 mg L$^{-1}$ for NO$_{2}$-N. The feeding was resumed, and the concentrations increased a second time to similar levels as before. The feeding was again stopped, and the concentrations declined again (0.4 mg L$^{-1}$ TAN and 0.08 mg L$^{-1}$ NO$_{2}$-N). A third increase in the concentrations was seen, TAN increased to 1.5 mg L$^{-1}$ and NO$_{2}$-N to 0.17, and the feeding was stopped again. At this point, the salinity was 12‰, and thereafter TAN and NO$_{2}$-N remained below 1.1 and 0.16 mg L$^{-1}$, respectively. The biofilter function was relatively stable when the salinity was > 12‰.

The average temperature in bRAS was significantly higher than in sRAS (Table 1) throughout the experiment, both during the acclimatization period ($p < 0.001, 2.6 ^\circ C$ higher) and during the salinity increase ($p < 0.001, 1.0 ^\circ C$ higher). The temperature in the sea (which sRAS got most of the water from) was lower than the water from the freshwater distribution system (which bRAS got most of the water from), causing the temperature in bRAS to be higher. The average concentrations of TAN and nitrite were significantly higher in sRAS than in bRAS through the experiment ($p < 0.05$). Even though TAN and NO$_{2}$-N peaks were observed during the salinity increase in bRAS (Fig. 2), the concentrations in sRAS were still on average higher. This shows that the nitrification efficiency was overall lower in sRAS with stable high salinity than in bRAS with increasing salinity.

3.2. Salinity dependent nitrification capacity of biofilter carriers from bRAS during adaptation to increasing salinity

The maximum ammonia oxidation capacities (AOR$_{\text{max}}$) of the carriers when the salinity in bRAS was 5‰ was inhibited 25% and 37% when stressed with a salinity of 12 and 28‰, respectively (Fig. 3). The reactor stressed with 28‰ had the highest reduction and the longest lag-phase of 90 min before nitrification could be observed (Supplemental S1). When the salinity was 12‰ in bRAS, no lag-phases were
observed in any of the reactors. The AORmax was inhibited by 40, 25 and 17% when stressed with 28, 20 and 5‰ salinity, respectively. When the salinity in bRAS was 20‰, the reactor stressed with 5‰ had the lowest AORmax (37% reduction) and longest lag-phase. The AORmax in the native salinity reactors (reactors holding the salinity that bRAS was operated with at that time) were similar in each capacity test, with around 0.40 g N m\(^{-2}\) d\(^{-1}\). When bRAS was at 12 and 20‰ salinity, the native reactors had the highest AORmax. These results show that the AOBs were affected negatively by deviations from the native bRAS salinity. When bRAS was at 5‰ salinity, the reactors stressed with higher salinity (20 and 28‰) had lag-phases, and when bRAS was at 20‰ salinity, the reactors stressed with lower salinity (5 and 12‰) had lag-phases and lower AORmax. More details on the regression analysis can be found in the Supplemental Table S1.

Regardless of salinity in bRAS, the concentration of NO\(_2\)-N throughout all the capacity tests was highest in the 28‰ salinity reactor and lowest in the 5‰ reactor (Fig. 4). Even though the AORmax in general decreased when the carriers were subjected to higher or lower osmotic stress than their native salinity, this was not reflected in the NO\(_2\)-N concentrations. The concentrations in the 28‰ salinity reactors at the end of the capacity tests were a factor 3.4, 3.0 and 5.6 higher than in the 5‰ reactors at 5, 12 and 20‰ native bRAS salinity, respectively. These results indicate that the AOBs are negatively affected by deviations from native salinity, whereas the NOBs are negatively affected by increased salinity. Due to high oxygenation of the reactors, possible denitrification was not included in the nitrification analysis.

3.3. Bacterial community composition

The Illumina sequencing yielded a total of 4255 OTUs from the raw data. The sequencing depth was on average 40,000 reads for water and biofilter biofilm samples. For faecal samples the number of reads was variable, ranging from 4000 to 80,000 reads. However, 50 to 90% of the reads in the faecal samples were classified as *Streptophyta*, and are likely from higher plants or algae used as feed ingredients. These reads were removed from the dataset before further analysis. After normalization and removal of plant/algal OTUs, there were a total of 3064 OTUs in the water, 1602 OTUs in the biofilter biofilm and 1480 OTUs in faecal samples.

### Table 1

Water quality variables (± SD) in bRAS and sRAS.

| Variable   | bRAS After split 3‰ | 3–26‰ Day −60 - 0 | Day 1–28 | sRAS After split 25‰ | 28‰ Day −60 - 0 | Day 1–28 |
|------------|----------------------|-------------------|----------|----------------------|-----------------|----------|
| Temperature (°C) | 16.1 ± 0.8\(^a\) | 15.2 ± 1.1\(^c\) |           | 13.5 ± 0.9\(^a\) | 14.2 ± 0.7\(^c\) |           |
| Salinity (‰) | 4.0 ± 1.4\(^c\) | Fig. 2 |           | 25.7 ± 3.2\(^a\) | 28.2 ± 0.7 |           |
| pH         | 7.0 ± 0.3           | 7.1 ± 0.6         |           | 7.1 ± 0.3           | 7.1 ± 0.2       |           |
| TAN (mg L\(^{-1}\)) | 1.1 ± 0.7\(^c\) | 0.8 ± 0.4 (Fig. 2) |       | 1.4 ± 0.9\(^a\) | 1.2 ± 0.3\(^c\) |           |
| NO\(_2\) – N (mg L\(^{-1}\)) | 0.1 ± 0.2\(^c\) | 0.1 ± 0.1\(^c\) (Fig. 2) |   | 0.3 ± 0.3\(^c\) | 0.2 ± 0.04\(^c\) | |

\(^a\) Statistically significant, \(p < 0.05\).
OTU, *Nitrospira* 15 OTUs and *Nitrolancea* 3 OTUs. However, only 2 out of the 21 nitrifying OTUs had a relative abundance higher than 0.1%, and these were included in Fig. 5. The *Nitrosomonas* OTU_77 and *Nitrospira* OTU_45 were present in both sRAS and bRAS, thus these nitrifying OTUs can be present both at low and higher salinity. In bRAS, as the salinity increased, the *Nitrospira* OTU_45 decreased in relative abundance from around 16% at Day 1 (3‰) to 10% on Day 19 (18‰), and by Day 28 (26‰) the abundance was 0.5%. The *Nitrosomonas* OTU_77 had a relative abundance of around 1% from 3 to 6‰ salinity, then it increased to 8% at 18‰ salinity. In sRAS, the relative abundance of *Nitrosomonas* OTU_77 was low throughout the experiment, around 1%, and *Nitrospira* OTU_45 had an abundance of 3–5%.

3.3.2. Bacterial community structures and succession in the water

To visualize the temporal development of the microbial communities in bRAS and sRAS water, ordination by Principal Coordinate Analysis (PCoA) based on Bray-Curtis similarities was performed. The ordination for the first two axes explained 57% of the variance in the dataset (Fig. 6A), and showed that the samples from sRAS and bRAS were fully separated on Axis 1. The samples from sRAS clustered throughout the experiment, but samples from the water going out of the biofilter were slightly separated from the water sampled from the fish tanks and water going in to the biofilter. For bRAS the samples were more spread and a succession of the microbial communities due to salinity increase can be observed through the experiment, primarily along Axis 2. Also in bRAS, the samples from the biofilter were separated from the fish tank samples. Examining the third axis of the ordination, (Fig. 6B) the samples in sRAS at the split of the systems (D-60) were clearly separated from the rest of the samples. One-way PERMANOVA confirmed that when samples were grouped by sampling day and treatment, all groups were significantly different ($p < 0.03$), including the samples of sRAS.

Bray-Curtis and Sørensen-Dice similarities were calculated to examine the succession of the microbial communities in the water between two succeeding sampling times within each system. In bRAS, comparing the samples at the split of the systems on Day $-60$ and Day 1 was the most dissimilar comparison of succeeding samples, for both Sørensen-Dice and Bray-Curtis (0.5 and 0.3, respectively). The salinity was the same for this period (3‰), however this comparison had the longest interval in days and a change in fish biomass in both systems. The Bray-Curtis similarities comparing salinities 3–6‰ and 6–12‰ were around 0.4. Then after 12‰ the comparisons increased to 0.6 and were approximately at the same level as Sørensen-Dice. This indicate that the change in abundance of the OTUs present up to 12‰ contributed more to the succession of the microbial communities than introduction of new OTUs. Whereas after 12‰ and up to 26‰, the succession is equally contributed by introduction of new OTUs and change in abundance of present OTUs. This furthermore
suggests that the OTUs with low abundance are replaced with new OTUs. Comparing the water samples in bRAS at Day 1 and Day 28 (3 vs 26‰) (Supplemental, Table S2), the Bray-Curtis similarity was around 0.3, and at the same value as when comparing Day −60 and Day 1. Even though the salinity was the same on Day −60 and Day 1 (3‰), the Bray-Curtis similarity is the same as when comparing the two sampling times with 8.7 times salinity increase. For sRAS (Supplemental S2B), the comparison between Day −60 and Day 1 was also the most dissimilar (Bray-Curtis: 0.37, Sørensen-Dice: 0.5). Salinity was a driver for succession, but other factors (e.g. change in fish biomass) also contributed to the change in bacterial community composition.

There were 1767 and 2171 OTUs in total found in bRAS and sRAS water, respectively. The number of OTUs that were shared in both systems was 874, constituting 50 and 40% of total OTUs in bRAS and sRAS, respectively. The most abundant OTUs in bRAS and sRAS (Fig. 7) were found in both systems. However, the general trend was that if the OTU was in high relative abundance in one system, it was low in the other. Examples of this was OTU_8, Gemmobacter. This OTU was highly abundant in bRAS and had very low abundance in sRAS. In bRAS OTU_8 decreased in relative abundance from 33% at Day −60 to 4% at Day 1 and then increased to 14% by Day 13 at 12‰ salinity. After 12‰ salinity, it decreased again to 5% by the end of the experiment. The relative abundance of OTU_196 (Pseudohodobacter) increased as the salinity increased from 3% to 22% through the experiment form Day 1 to 28. The opposite was observed for OTU_13, Mycobacterium. This OTU decreased in relative abundance from 19% to 3% as the salinity increased through the experiment. Loktanella (OTU_3) had very low abundance in bRAS at salinity 3–12‰ salinity, however at 18‰ and 26‰ salinity the abundance increased to 2 and 4%, respectively. Loktanella is highly abundant in sRAS (from 7 to 30% through the experiment), thus Loktanella succeeds with salinities higher than 12‰. Similar trends are seen for Leucothrix (OTU_16) as it increased from 1 to 7% in relative abundance in bRAS from 18‰ to 26‰ salinity and had a relative abundance in sRAS from 4 to 10%.

The OTU richness in bRAS at each sampling and the number of shared OTUs between sampling times are presented in Table 2. On Day 1 (3‰), the total number of OTUs were 1087, and as the salinity increased up to 26‰, there was a decrease in the number of shared OTUs from Day 1 to Day 28. Out of the total OTUs present on Day 1, 47.9% were still present on Day 28 with 26‰ salinity. From Day −60 to Day 1, the total number of OTUs increased a factor 1.73. On Day −60 half the fish group was moved to sRAS. This shows that a reduction in fish biomass and organic loading, increased the total number of OTUs. In
sRAS the opposite was observed, and the total number of OTUs decreased a factor 1.4 from Day −60 (1481 OTUs) to Day 1 (1051 OTUs) as 100,000 fish were stocked in the system.

The taxa summary of bacterial orders in the water samples (Supplemental S3) showed a change in the relative abundance of the taxa in bRAS through the experiment, whereas for sRAS there was less variation. Going from Day −60 to Day 1 in bRAS, the salinity was the same (3‰), but a change in the most abundant orders Rhodobacteriales, Thiotrichales, Burkholderidales and Actinomycetales are evident. In the fish tanks, Rhodobacteriales and Thiotrichales decreased in relative abundance from 40 to 50% and 20–25% to 10% and 2–10%, respectively. Burkholderidales and Actinomycetales increased in relative abundance from 3 to 5% to 10–15%. The bacterial communities remained at these relative abundances during the salinity increase from 3 to 6‰ (Day 1–6). On Day 13 (12‰) and throughout the salinity increase to Day 28 (26‰), the relative abundance of Rhodobacteriales (40–50%), Thiotrichales (10%), Burkholderidales (5–30%) and Actinomycetales (3–5%) remained relatively stable even though the salinity more than doubled (Marked in red: Supplemental S3). The samples from the water going out of the biofilter differentiated from the fish tanks and biofilter in samples, which was evident for both systems. In sRAS, the most abundant orders were Rhodobacteriales and Flavobacteriales, and less change in the relative abundance were observed here compared to bRAS which coincides with the PCoA-plot (Fig. 6).

### 3.3.3. Faecal microbiota

The faecal microbiota of the individuals within the fish tanks had a Bray-Curtis similarity of around 0.5 in bRAS and 0.5–0.6 in sRAS on Day 28, when salinity was 26–28‰ (Fig. 8A). The faecal microbiota of fish in the sea cages had a lower Bray-Curtis similarity in both systems, with 0.22 and 0.30 for the bRAS and sRAS group, respectively. As the salinity increased in bRAS, the faecal microbiota of the individuals became more similar to the faecal microbiota of the sRAS individuals and increased from 0.2 at 3‰ to 0.5 at 26‰ salinity (Fig. 8B). The Bray-Curtis similarity of the individuals within a fish tank on Day 28 and the similarity between bRAS and sRAS the same day were similar and around 0.5. This means that the variation in faecal microbiota between individuals within a fish tank was comparable to the variability between the two different systems that had different water

![Fig. 7. Most abundant OTUs in the fish tank water of A) bRAS and B) sRAS. All OTUs are classified for the genus with a confidence threshold of 0.8.](image-url)
frequency (number of fish) in each weight range was significantly lower than in bRAS. The fish grew better than in sRAS during the acclimatization period after being transported to sea water, but the appetite was reduced during the salinity increase period. The opposite could be seen by a nitrifier specialized for that salinity. The concentrations of NO₂-N in the reactors were always lowest in the 5‰ reactor, regardless of the salinity increase. However, the AORcapacity tests showed that when the carriers were stressed with higher or lower salinity than the native bRAS salinity, the AORmax was lower than 1.0 °C lower in sRAS (Table 1).

The higher growth in bRAS can be explained by the higher temperatures and also the sRAS fish had reduced appetite though the temperature was on average 1.0 °C lower in sRAS (Table 1).

Comparing the faecal microbiota of the fish before and after sea transfer, the Bray-Curtis similarity was 0.26 ± 0.10 and 0.30 ± 0.12 for bRAS and sRAS, respectively. A t-test confirmed that the difference was not significant (p = 0.11), thus both the fish groups changed the faecal microbiota to the same degree after sea transfer.

### 3.4. Performance of fish

After the split of the systems, the fish in sRAS were 19% bigger (p < 0.001) than the fish in bRAS (Table 3). During the acclimatization period (From Day −60 to Day 1), fish in sRAS grew a factor 2.2 and in bRAS a factor 2.6. The higher growth in bRAS can be explained by the higher temperatures and also the sRAS fish had reduced appetite after having been moved. On Day 1 when the salinity was increased, the fish in bRAS and sRAS started out with comparable weight (p = 0.56). At the end of the salinity increase period (Day 28) the fish in sRAS were 14% and significantly (p < 0.001) bigger than the fish in bRAS even though the temperature was on average 1.0 °C lower in sRAS (Table 1). From Day −60 to Day 28, the fish in bRAS and sRAS grew 208 and 231 g, respectively. The sRAS fish had lower growth in the acclimatization period after being transported to sea water, but the appetite was resumed during the salinity increase period. The opposite could be seen for bRAS. The fish grew better than in sRAS during the acclimatization period, however during the salinity increase the fish growth was reduced (despite higher temperatures). After the sea cage phase, the frequency (number of fish) in each weight range was significantly different between the bRAS and sRAS group (p < 0.0001). The average weight of the sRAS group was 2.6% bigger than in bRAS due to less individuals with weight < 4 kg (15 vs 10%). Both fish groups were diagnosed with the cardiac disease Cardiomyopathy syndrome (CMS), and the disease was observed both on land and at sea. The fish in both groups showed normal behaviour except for reduced appetite at the mentioned time periods. The mortality was 3.9 and 1.1 times higher in the sRAS group than the bRAS group on land and at sea, respectively. Thus, the fish in sRAS grew slightly better in total, but had a 1.5 times higher mortality in total and produced less biomass.

### 4. Discussion

Two regimes for transition from brackish to seawater in production of Atlantic salmon post-smolt was studied holistically, by gathering data on nitrification, composition of heterotrophic and nitrifying bacteria throughout the system and data on performance and gut microbiota of the fish.

#### 4.1. The same nitrifying OTUs were found in both systems, and a low AOR:NOB ratio suggests presence of comammox

In bRAS, the nitrifying bacteria present in the biofilter at 12‰ were able to handle the osmotic stress of water with salinity up to 26‰, without compromising the efficiency in nitrification (Fig. 2). The capacity tests showed that when the carriers were stressed with higher or lower salinity than the native bRAS salinity, the AORmax was lower than in the native salinity reactors (Fig. 3). The inhibition varied between 25 and 40%, which is lower than what have been seen in other studies with salt stress in freshwater nitrifying reactors (97–100% inhibition: Gonzalez-Silva et al., 2016; Kinyage et al., 2019). Historically, the bRAS system had always been operated with some salinity (3–5‰ salinity 6 months prior to our experiment), and also at higher salinities (20‰ for 3 years) for earlier production groups at the facility. Seawater priming have shown to improve salinity acclimatization (Navada et al., 2020), thus if bRAS had been primarily a strict freshwater (0‰) biofilter with no previous experience of osmotic stress, a higher inhibition might have been seen. As the lag-phases were only 10–90 min, depending on the stress-level, the nitrifying bacteria present must have adapted physiologically to the new salinity rather than being replaced by a nitrifier specialized for that salinity. The concentrations of NO₂-N in the reactors were always lowest in the 5‰ reactor, regardless of the native bRAS salinity. This indicate that nitrite oxidizers were more affected by salinity increase than ammonia oxidizers. However, the concentrations of nitrite were relatively low (< 0.5 mg L⁻¹) in all the reactors, and in bRAS NO₂-N did not accumulate after the salinity had increased.
reached 12‰.

Only two OTUs identified as nitrifying bacteria were found in relative abundance over 0.1% in the biofilm. Both these OTUs were found in both systems, thus they functioned at both low and higher salinities. The abundance of *Nitrosomonas* was low, especially in sRAS and in the beginning of the experiment in bRAS. *Nitrospira* was more abundant in both systems, except for the last sampling in bRAS. *Nitrosomonas* are AOBs, and a low AOB:NOB ratio have been suggested as an indicator of presence of complete ammonia oxidation (comammox) bacteria (Fowler et al., 2018). *Nitrospira* was previously thought to be nitrite oxidizing bacteria, however recent studies have found that some *Nitrospira* species are comammox bacteria and oxidize both ammonia and nitrite (van Kessel et al., 2015; Daims et al., 2015). Comammox *Nitrospira* have been found in biofilters treating waters with low concentration of ammonia, like RAS and ground water (van Kessel et al., 2015; Gülay et al., 2016). Comammox nitrifiers could therefore be in the nitrifying consortium in the systems studied. OTU_77 had 94–96% sequence match with three *Nitrosomonas* species which have been reported to require salt for growth, *N. aestuarii*, *N. halophila* and *N. marina* (Koops et al., 1991). Both bRAS and sRAS got the same intake water, but in different ratios to achieve the respective salinities, thus the same bacteria had been introduced to both systems and biofilter. Because both OTUs were present in bRAS (and throughout the salinity increase) and sRAS, it suggests that these nitrifying OTUs can adapt to salinity in the range from 3 to 26‰, and also 28‰.

**4.2. Increasing the salinity beyond 12% did not change the bacterial community compositions of bRAS water**

By increasing the salinity approximately 1% per day, a succession in the bacterial communities was observed in the water samples in bRAS (Fig. 6 and Supplemental S3). There were, however, also observed changes in the community structures when the salinity was constant in the acclimatization period. In this period, bRAS had a reduction of the organic load by moving 100,000 fish to sRAS. Whereas sRAS got a sudden and high organic load with the introduction of 100,000 fish. An increase in organic load decreased the OTU richness, whereas a relief in organic load increased the richness (Table 2). Organic matter is substrate for heterotrophic bacteria in RAS (Michaud et al., 2006), and changes in community compositions due to changes in organic matter loading have been seen in other studies (Wold et al., 2014; Fossmark et al., 2020). In bRAS, increasing the salinity from 6 to 12‰, the change in microbial community dynamics was higher than increasing it above 12‰ (Higher Bray-Curtis: Supplemental S2). It seems like there is a threshold salinity somewhere between 6 and 12‰ because increasing the salinity 2.2 times from 12 to 26‰ did not induce large changes in the community structures. The same trends have been seen in hierarchical clustering based on bacterial community composition similarities in the Baltic Sea (Herlemann et al., 2011). In that study there were clustering of samples in three salinity ranges: 0–3.2‰, 4.6–7.7‰ and 10.5–30.9‰. Herlemann et al. (2011) concluded that the OTUs present at brackish water localities were not specialized for brackish water, but adapted bacteria originating from marine and freshwater environments. In our study, half of the OTUs present in the water at 3‰ also adapted to the higher osmotic stress during the salinity increase to 26‰ in bRAS (Table 2). The OTUs with high abundance were generally the ones to adapt, whereas the OTUs with low abundance were replaced by new OTUs. This density dependent ability to adapt, suggests that low density OTUs are vulnerable to drift during the adaptation period.

The most abundant genera in sRAS were in general in low abundance in bRAS, and vice versa (Fig. 7). The OTU with highest abundance in sRAS was OTU_3 *Loktanella*. This OTU got the highest sequence match of 98.8% with the species *Loktanella acticola* (Park et al., 2017). Park et al. (2017) reports that this species grew optimally from 20 to 30‰ salinity. This corresponds well with the findings in our experiment as OTU_3 was found in higher abundance in bRAS at 18% to 26‰, and high relative abundance (7–30%) in sRAS. This OTU is thus selected for at higher salinities. *Loktanella maritima*, which have shown to be commensal bacterium in production of lobster, and inhibit the growth of pathogenic *Vibrio parahaemolyticus* (Ranson et al., 2018). Another species in this genus, *Loktanella koreensis*, is an algicidal bacterium which have shown to be important in controlling proliferation of algae (Meyer et al., 2017). Some species of this genus thus have symbiotic/commensal interactions in different marine ecosystems. In sRAS *Leucothrix* OTU_16 was also highly abundant, and got 93.2% sequence match with the filamentous bacteria *Leucothrix mucor* (Ludwig et al., 1995). This species have been found to densely colonize cod eggs (Hansen and Olafsen, 1989) and was thought to negatively affect the embryo development due to hypoxia conditions caused by the bacteria. *Leucothrix* was also found in cultivation of lump fish in RAS, and were hypothesized to be a cause of poorer gill health of the fish (Dahle et al., 2020). *Leucothrix* could therefore have a negative effect on the bacterial water quality in RAS. The OTU with the highest abundance in the dataset was OTU_8, *Gemmobacter*, and was found in bRAS in high abundance. Little is known about this genus and for OTU_8 the highest sequence match was 98.8% with *Gemmobacter tilapiae* (Sheu et al., 2013), a strain isolated from a fresh water pond with Tilapia fish (*Tilapia rendalli*). Sheu et al., 2013 did not report any disease of the fish or other effects associated with this bacterial genus. OTU_13 *Mycobacterium* had 90.1% match with several different *Mycobacterium* species. The known salmon pathogen *Mycobacterium salmoniphilum* which causes mycobacteriosis in Atlantic salmon (Aro et al., 2014) did not match the representative sequence found in this experiment. Other genera known to be potentially pathogenic in salmon farming was not found in high abundance and were not studied further.

**4.3. Salinity drove the succession of the faecal microbiota, and high inter-individual variation within the fish tanks suggests that stochastic processes also affect the succession**

The faecal microbiota of individuals in bRAS evolved to become more similar to sRAS individuals as the salinity was increased (Fig. 8). Even though the water microbiota was very similar in the different fish tanks (Fig. 6 and Supplemental Table S2 Average Bray-Curtis: 0.86), this was not reflected in the faecal microbiota between individuals within a fish tank or between fish tanks within a system. This is emphasized by the fact that the similarity between individuals within a fish tank was the same as the similarity between the two RAS at Day 28 (Bray-Curtis: 0.5, Fig. 8). The same was seen when comparing the microbiota of individual fish within and between the sea cages. This shows that there are other factors than water microbiota involved in the assembly of the gut microbiota of Atlantic salmon smolt. High inter-individual differences in the gut microbiota have been seen in other studies of farmed Atlantic salmon in the same freshwater rearing systems (Dehler et al., 2017), and in farmed and wild Atlantic cod caught at the same location (Fjellheim et al., 2012; Star et al., 2013; Bakke et al., 2015). Dehler et al. (2017) and Star et al. (2013) found core OTUs that were consistently seen in all individuals, and it was discussed that the core OTUs could be important for the fish health and that they are actively selected for in the host. The OTUs who’s presence differ between individuals may have redundant functions, and their presence is explained by stochasticity (Zhou and Ning, 2017). The first colonization of the gut of fish larvae could also have an impact on the gut microbiota for juvenile/adult stages, so-called priority effect (De Schryver and Vadstein, 2014). The order of species colonizing an environment is suggested to cause divergence between communities even though the environmental conditions are the same (Nemergut et al., 2013). The fish in our study were smolt and in a critical stage in their life cycle by their preparation for a life in seawater. This causes stress, which has shown to alter the intestinal lining of salmon and furthermore affect the
number of bacteria in the faeces (Olsen et al., 2002). Clearly, many factors affect the succession of the gut and faecal microbiota of salmon smolt, and salinity has shown to be one driver for the succession. It has been hypothesized that a gradual succession in the gut microbiota is better for fish health than sudden abrupt changes (De Schryver and Vadstein, 2014). After sea transfer, the faecal microbiota in both fish groups changed to the same degree (same Bray-Curtis values comparing microbiota before and after sea transfer), regardless of the salinity regime operated on land. To what extent the different salinity regimes had an effect on the faecal microbiota after transfer to sea is therefore hard to conclude on. More samples later in the sea cage phase should have been collected, to see if there were any long-term effects on the different salinity regimes.

4.4. Did the different salinity regimes affect fish growth and mortality? Both fish groups were diagnosed with CMS, the fish from the sRAS group grew better than the bRAS group, however the sRAS fish had higher mortality. On land, the highest mortality of the sRAS fish was observed when the fish had a high growth rate. This was 4 weeks after the fish were moved to higher salinity and when the appetite was resumed. CMS affects the cardiovascular capacity (Garseth et al., 2018) and have shown to affect fish with fast growth and high condition factor (Løvoll et al., 2010). Therefore, the better growth in sRAS could in fact be the reason why more fish in this group were affected by CMS. It could also be that the sRAS fish were more susceptible to the disease due to stress caused by moving the fish from bRAS to sRAS at the split of the fish group, and sudden change to high salinity. It is hard to conclude whether the different salinity regimes on land affected the mortality and growth rates of the fish or not. The hypothesized positive effects of acclimatizing the microbiota associated with the water and salmon gut to higher salinity before sea transfer can therefore not be supported, as there is much uncertainty due to the CMS and what caused the RAS-groups to have different mortality and growth rates. Excluding the mortality and only including the fish weights at the end, the hypothesis is contradicted as the fish in sRAS grew better, both on land and in the sea. In the faecal microbiota, it seems like other factors such as stochastic processes and stress may undermine the effects of salinity matura-tion. The gradual salinity increase induced peaks in TAN and NO2-N concentrations in bRAS, however the average concentrations in sRAS were still significantly higher during the experiment. Why TAN and NO2-N were higher in sRAS with stable salinity could be the introduction of the 100,000 fish which increase the organic load, which have shown to negatively affect nitrification (Michaud et al., 2006; Guerdart et al., 2011). The relative abundance of nitrifiers were also lower for the sRAS biofilter, in addition to the lower temperature which could have affected the nitrification efficiency negatively. It has been shown that salmon parr exposed to TAN concentrations of up to 25 mg L\(^{-1}\) (35 \(\mu\)g L\(^{-1}\) NH\(_4\)-N) did not affect the growth or welfare of the fish (Kolarvic et al., 2013). Therefore, the difference in mortality and growth between the systems were probably not attributed to the differences in nitrification in the systems, as the concentrations of bRAS and sRAS were 20 times below this concentration. Nevertheless, we can conclude from this study is that increasing the salinity by 1% per day is possible in a RAS with salmon post-smolt without exceeding toxic concentrations of TAN and NO\(_2\)-N. However, we believe this is valid if the RAS and biofilter has experienced osmotic stress earlier and will not be true for a strict freshwater system where the inhibition of nitrification probably will be higher. The history of the biofilter must be taken into consideration for fish farmers when planning to increase the salinity in an operating RAS with fish.

5. Conclusion Salinity has shown to be a driver of succession in RAS, and other factors such as organic load in the water and stochastic processes in the host also affected the development of bacterial communities. We found indications for a threshold salinity between 6 and 12‰, whereafter the bacterial community compositions in the water were stable to sub-sequent salinity increase. The biofilters in bRAS and sRAS had the same dominating nitrifying OTUs which were able to adapt to a salinity increase from 3 to 26 and 28‰. The capacity tests showed that the AORmax was inhibited up to 40% when the carriers were stressed with higher or lower salinity than the native bRAS salinity. Increasing the salinity around 1‰ per day in RAS for salmon post-smolt production is possible if the biofilter have some history with osmotic stress. The fish in bRAS had a lower mortality than the fish in sRAS, however the sRAS group grew better.

Declaration of Competing Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements This study was funded by Regional Forskningsfond Nord (RFF Nord), Skatte Funn, LetSea AS and NTNU Oceans and NTNU Faculty of Engineering. The NTNU funding was a scholarship to ROF. The authors would like to thank the staff at Let Sea for all the help and practical support during the experiment. Special thanks to Vegbørn Ulvang, Theis Bue Christensen, Espen Eide and Bjørn Erik Sørvig. Anette V. Bugten is thanked for all the help during sampling at Dønna.

References Abolofia, J., Asche, F., Wilen, J.E., 2017. The cost of lice: quantifying the impacts of Parasitic Sea lice on farmed Salmon. Mar. Resour. Econ. 32 (3), 329–349. https://doi.org/10.1086/691981.
Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215 (3), 403–410. https://doi.org/10.1016/S0022-2836(05)80260-2.
Anderson, M.J., 2017. Permutational multivariate analysis of variance (PERMANOVA). In: Balakrishnan, N., Colton, T., Everitt, W., Piegorch, F., Ruggeri, F., Teugels, J.L. (Eds.), Wiley StatsRef: Statistics Reference Online.
Ars, L., Corea, K., Martinez, A., Ildéfonso, R., Yáñez, J.M., 2014. Characterization of Mycobacterium salmoniphilum as causal agent of mycobacteriosis in Atlantic salmon, Salmo salar L., from a freshwater recirculation system. J. Fish Dis. 37 (4), 341–348. https://doi.org/10.1111/jfd.12108.
Arlen, S., Simsek, E., 2012. Influence of salinity on partial nitrification in a submerged biofilter. Bioreour. Technol. 118, 24–29. https://doi.org/10.1016/j.biortech.2012.05.057.
Bakke, I., Coward, E., Andersen, T., Vadstein, O., 2015. Selection in the host structures the microbiota associated with developing cod larvae (Gadus morhua). Environ. Microbiol. 17, 10. https://doi.org/10.1111/1462-2920.12888.
Bakke, I., Am, A.L., Kolarevic, J., Yrestoyl, T., Vadstein, O., Attramadal, K.J.K., Terjesen, B.F., 2017. Microbial community dynamics in semi-commercial RAS for production of Atlantic salmon post-smolts at different salinities. Aquac. Eng. 78, 42–49. https://doi.org/10.1016/j.aquaeng.2016.10.002.
Bassin, J.P., Kleerebezem, R., Muizer, G., Rosado, A.S., van Loosdrecht, M.C.M., Dezotti, M., 2012. Effect of different salt adaptation strategies on the microbial diversity, activity, and settling of nitrifying sludge in sequencing batch reactors. Appl. Microbiol. Biotechnol. 93 (3), 1281–1294. https://doi.org/10.1007/s00253-011-3428-7.
Blancheton, J.P., Attramadal, K.J.K., Michaud, L., d’Orbcastel, E.R., Vadstein, O., 2013. Insight into bacterial population in aquaculture systems and its implication. Aquac. Eng. 53, 30–39. https://doi.org/10.1016/j.aquaeng.2012.11.009.
Chao, A., Chazdon, R.L., Colwell, R.K., Shen, T.J., 2006. Abundance-based similarity indices and their estimation when there are unseen species in samples. Biometrics 62 (2), 361–371. https://doi.org/10.1111/j.1541-0429.2005.00489.x.
Chen, S.L., Ling, J., Blancheton, J.P., 2006. Nitrification kinetics of biofilm as affected by water quality factors. Aquac. Eng. 34 (3), 179–197. https://doi.org/10.1016/j.aquaeng.2005.09.004.
Cole, J.R., Wang, Q., Fish, J.A., Chai, B., McGarrell, D.M., Sun, Y., Brown, C.T., Porras-Alfaro, A., Kuske, C.R., Fjie, J.M., 2013. Ribosomal database project database: data and tools for high throughput rRNA analysis. Nucleic Acids Res. 42 (D1), D633–D642. https://doi.org/10.1093/nar/gkt1264.
Corrêa-Lorenzo, C., Rodríguez-Díaz, M., Sipkema, D., Juárez-Jiménez, B., Rodelas, B., Smith, H., González-López, J., 2015. Effect of salinity on nitrification efficiency and structure of ammonia-oxidizing bacterial communities in a submerged fixed bed bio reactor. Chem. Eng. J. 266, 233–240. https://doi.org/10.1016/j.cej.2014.12.003.
Dahle, S.W., Bakke, I., Birkeland, M., Nordøy, K., Dalum, A.S., Attramadal, K.J.K., 2020. Production of lumpfish (Cyclopterus lumpus L.) in RAS with distinct water treatments: effects on fish survival, growth, gill health and microbial communities in rearing water and bioculum. Aquaculture 522, 10735097.

Daims, H., Lebedeva, E.V., Pjvach, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinusky, M., Vierheilig, J., Bulaev, A., Kirkegaard, R.H., van Bergen, M., Rattie, T., Bendinger, B., Nielsen, P.H., Wagner, M., 2015. Complete nitrification by Nitrosomonas bacteria. Nature 528 (7584), 504–509. https://doi.org/10.1038/nature16461.

Dalgaard, J., LUND, L., THORARSINN, D., DRANGSTI, A., ARVONEN, K., PEDERSEN, P.B., PEDERSEN, L.-F., 2019. Effects of abrupt salinity increase on freshwater reared Atlantic salmon (Salmo salar) during seawater transfer. Aquaculture 423 (1–4), 154–164. https://doi.org/10.1016/j.aquaculture.2014.09.025.

Dahle, S.W., Bakke, I., Birkeland, M., Nordøy, K., Dalum, A.S., Attramadal, K.J.K., 2020. Production of lumpfish (Cyclopterus lumpus L.) in RAS with distinct water treatments: effects on fish survival, growth, gill health and microbial communities in rearing water and bioculum. Aquaculture 522, 10735097.

Daims, H., Lebedeva, E.V., Pjvach, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinusky, M., Vierheilig, J., Bulaev, A., Kirkegaard, R.H., van Bergen, M., Rattie, T., Bendinger, B., Nielsen, P.H., Wagner, M., 2015. Complete nitrification by Nitrosomonas bacteria. Nature 528 (7584), 504–509. https://doi.org/10.1038/nature16461.

Dalgaard, J., LUND, L., THORARSINN, D., DRANGSTI, A., ARVONEN, K., PEDERSEN, P.B., PEDERSEN, L.-F., 2019. Effects of abrupt salinity increase on freshwater reared Atlantic salmon (Salmo salar) during seawater transfer. Aquaculture 423 (1–4), 154–164. https://doi.org/10.1016/j.aquaculture.2014.09.025.
2018. Draft genome sequence of *Loktanella maritima* strain YPC211, a commensal bacterium of the American lobster (*Homarus americanus*). Genome Announc. 6 (18), e00314–e00318. https://doi.org/10.1128/genomeA.00314-18.

Rud, I., Kolarevic, J., Holan, A.B., Berget, I., Calabrese, S., Terjesen, B.F., 2017. Deep-sequencing of the bacterial microbiota in commercial-scale recirculating and semi-closed aquaculture systems for Atlantic salmon post-smolt production. Aquac. Eng. 78, 50–62. https://doi.org/10.1016/j.aquaeng.2016.10.003.

Rudi, K., Angeli, I.L., Pope, P.B., Vik, J.O., Sandve, S.R., Snipen, L.-G., 2018. Stable core gut microbiota across the freshwater-to-salinity transition for farmed Atlantic Salmon. Appl. Environ. Microbiol. 84 (2). https://doi.org/10.1128/aem.01974-17. e01974-17.

Rusten, B., Eikebrokk, B., Ulgenes, Y., Lygren, E., 2006. Design and operations of the Kaldnes moving bed biofilm reactors. Aquac. Eng. 34 (3), 322–331. https://doi.org/10.1016/j.aquaeng.2005.04.002.

Shapiro, S.S., Wilk, M.B., 1965. An analysis of variance test for normality (complete samples). Biometrika 52 (3/4), 591–611. https://doi.org/10.2307/2333709.

Sheu, S.-Y., Sheu, D.-S., Sheu, F.-S., Chen, W.-M., 2013. *Gemmobacter tilapiae* sp. nov., a poly-β-hydroxybutyrate-accumulating bacterium isolated from a freshwater pond. Int. J. Syst. Evol. Microbiol. 63 (4), 1550–1556. https://doi.org/10.1099/ijs.0.04735-0.

Star, B., Haverkamp, T.H.A., Jenoft, S., Jakobsen, K.S., 2013. Next generation sequencing shows high variation of the intestinal microbial species composition in Atlantic cod caught at a single location. BMC Microbiol. 13 (1), 248. https://doi.org/10.1186/1471-2180-13-248.

Uygur, A., Karg, F., 2004. Salt inhibition on biological nutrient removal from saline wastewater in a sequencing batch reactor. Enzym. Technol. 34 (3), 313–318. https://doi.org/10.1016/j.enzymtech.2003.11.010.

van Kessel, M.A.H.J., Speth, D.R., Albertsen, M., Nielsen, P.H., Op den Camp, H.J.M., Kartal, B., Jetten, M.S.M., Lücker, S., 2015. Complete nitrification by a single microorganism. Nature 528 (7583), 555–559. https://doi.org/10.1038/nature16459.

Wang, Z., Gao, M., She, Z., Wang, S., Jin, C., Zhao, Y., Yang, S., Guo, L., 2015. Effects of salinity on performance, extracellular polymeric substances and microbial community of an aerobic granular sequencing batch reactor. Sep. Purif. Technol. 144, 223–231. https://doi.org/10.1016/j.seppur.2015.02.042.

Wold, P.-A., Holan, A.B., Oie, G., Attramadal, K., Bakke, I., Vadstein, O., Leiknes, T.O., 2014. Effects of membrane filtration on bacterial number and microbial diversity in marine recirculating aquaculture system (RAS) for Atlantic cod (*Gadus morhua* L.) production. Aquaculture 422, 69–77. https://doi.org/10.1016/j.aquaculture.2013.11.019.

Zahran, H.H., 1997. Diversity, adaptation and activity of the bacterial flora in saline environments. Biol. Fertil. Soils 25 (3), 211–223. https://doi.org/10.1007/s003740050206.

Zhou, J.Z., Ning, D.L., 2017. Stochastic community assembly: does it matter in microbial ecology? Microbiol. Mol. Biol. Rev. 81, 4. https://doi.org/10.1128/mmbir.00002-17.