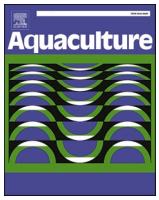
Aquaculture 565 (2023) 739155

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



Long-term microbial community structures and dynamics in a commercial RAS during seven production batches of Atlantic salmon fry (*Salmo salar*)

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ARTICLE INFO

Keywords:
Recirculating aquaculture systems
Microbial dynamics
Atlantic salmon
Water microbiota
Biofilm microbiota

ABSTRACT

The microbiota of recirculating aquaculture systems (RAS) is of major importance for optimal fish health. However, the microbial communities in commercial RAS are highly complex and more knowledge is needed to potentially control and maintain beneficial microbial communities for good fish production. In this study we monitored microbial communities in a commercial RAS producing Atlantic salmon fry (*Salmo salar*) during seven consecutive production batches. The water of rearing tanks and the water sump downstream of the biofilter/upstream of the UV, as well as biofilm of the wall of the rearing tanks and the fixed bed biofilter were analysed using 16S rRNA gene amplicon sequencing to elucidate the spatial-temporal microbial dynamics. The results showed that the microbiota composition of water and biofilm varied within and between the production batches, and that the fallowing periods had a substantial effect on the microbial communities. The correlation of the water and biofilm microbiota to fish presence in the system was confirmed by supervised machine learning. Shifts in the composition of the microbiota were identified in conjunction with variations in organic matter loading both during production and fallowing. In addition, variables like oxygen saturation, biomass, and feed type, showed good correlation with variations in the water microbiota composition. Although microbiota changed at fallowing, the microbiota returned to similar compositions during the production phases and was especially evident for the water microbiota. This indicates that the development of microbiota composition is strongly dictated by the similar selection pressure in the system. Nitrifying communities were dominated by *Nitrospira*, and the third most abundant *Nitrospira* OTUs were related to the comammox *Nitrospira nitrificans*. The microbial communities in the biofilter biofilm and water were significantly different but shared abundant taxa and followed the same temporal microbial dynamics and indicates an interaction between the biofilter biofilm and the suspended bacteria. CFU analysis showed that the fraction of rapid-growing bacteria was significantly higher in the rearing water than in the water sump upstream the UV disinfection, indicating that disinfection upstream the rearing tanks allowed for growth of opportunistic bacteria. A community with considerable potential for opportunistic regrowth can have consequences for the microbial water quality and the resistance against pathogen invasion The absence of an in-line disinfection step or placing the disinfection unit upstream the biofilter might provide better microbial water quality and a more resilient system against pathogen proliferation.

1. Introduction

Recirculating aquaculture systems (RAS) are increasingly being used for Atlantic salmon (*Salmo salar*) production (Badiola et al., 2012; Dalsgaard et al., 2013; Kolarevic et al., 2014; Davidson et al., 2017) due to the possibility of intensifying production while at the same time controlling the culture environment with minimal water usage and environmental impact (Martins et al., 2010; Dalsgaard et al., 2013; Davidson et al., 2017). The theoretical possibility of offering optimal environmental conditions means that the fish can obtain optimal

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https://doi.org/10.1016/j.aquaculture.2022.739155
Received 11 June 2022; Received in revised form 25 November 2022; Accepted 12 December 2022
Available online 15 December 2022

growth, survival, and disease resistance in RAS, provided technology and operation are fully mastered (Blancheton et al., 2013).

Microbes are ubiquitous and represent everything from an absolute necessity to a potential threat to life in RAS production. The biofilter is a central component in RAS and typically harbours a diverse microbiota, including nitrifying bacteria. RAS operation depend on nitrifying bacteria to convert toxic nitrogenous waste products from the fish to less toxic nitrate (Martins et al., 2010; Bartelme et al., 2017). Nitrification is a two-step process performed by ammonia oxidising bacteria (AOB) and ammonia oxidising archaea (AOA) that convert ammonia to nitrite, and nitrite oxidising bacteria (NOB) that convert nitrite to nitrate. Also, some bacteria can perform complete ammonia oxidation (comammox) in RAS (van Kessel et al., 2015). Denitrifying bacteria can be used in another treatment step to reduce water usage even further by converting nitrate into nitrogen gas that can be removed from the system (van Rijn et al., 2006). Different bacteria in nitrifying biofilters of RAS have been reviewed (Michaud et al., 2006; Schreier et al., 2010; Rurangwa and Verdegem, 2014; Ruan et al., 2015; Navada et al., 2019; Roalkvam et al., 2020; Navada et al., 2020a; Navada et al., 2020b; Fossmark et al., 2021; Bartelme et al., 2017), but the knowledge on temporal dynamics in commercial RAS is still scarce (Rojas-Tirado et al., 2019). Interaction and colonization with bacteria are essential for a normal and healthy development of the immune and digestive system of the fish (Llewellyn et al., 2014). In addition, a healthy host microbiota, as well as a beneficial and stable system microbiota, are thought to provide effective barriers against infection and development of disease (Vadstein et al., 2013). On the negative side, heterotrophic bacteria degrading organic matter increase oxygen consumption and waste loading on the system. High supply of available organic matter result in heterotrophic bacteria outcompeting the nitrifying bacteria and reduces the nitrification efficiency of the biofilter (Zhu and Chen, 2001; Michaud et al., 2006; Michaud et al., 2009; Schreier et al., 2010). Under specific conditions, several different species of microorganisms can produce by-products like toxic H_2S or off-flavour compounds, which can create problems in RAS (Guttman and van Rijn, 2008; Letelier-Gordo et al., 2020). In some cases, specific pathogenic species of bacteria can cause infections of the fish (Blancheton et al., 2013). However, a more common problem is the development of secondary infections of a weakened host by opportunistic bacteria (Vadstein et al., 2018).RAS have properties that can promote microbial stability and mutualistic fish-microbe interactions (Attramadal et al., 2014; Vadstein et al., 2018). The large surface area available for bacteria, the relatively stable organic loading, and the extended total hydraulic retention time of RAS creates strong competition between the bacteria. Strong competition for limited resources selects for a stable community dominated by slowly growing specialists at the expense of opportunists (Vadstein et al., 1993; Attramadal et al., 2012a, 2014; Vadstein et al., 2018; Vestrum et al., 2018; Attramadal et al., 2021). Also, the highly reduced amount of intake water increases the possibility of maintaining a high biosecurity into the RAS (Blancheton et al., 2013).The microbial communities in RAS can respond rapidly to changes in the environment (Bentzon-Tilia et al., 2016) with different selection pressures acting on the microbial communities. Different forces driving the selection pressure is feed and feeding regimes, the make-up water, management routines, system design, physicochemical water quality, and the fish itself (Attramadal et al., 2012a; Blancheton et al., 2013; Bakke et al., 2017; Rud et al., 2017; Vadstein et al., 2018; Fossmark et al., 2020; Fossmark et al., 2021; Dahle et al., 2020; Dahle et al., 2022; Almeida et al., 2021). Solutions to maintain beneficial microbial communities in RAS, which is important for system management and control, are practically lacking (Blancheton et al., 2013; Bentzon-Tilia et al., 2016).

In this study we characterized the microbiota of water and biofilm samples from a commercial RAS for production of Atlantic salmon fry for seven consecutive production batches. Samples were taken at six positions in the RAS loop every second week for 15 months. The six positions included the rearing water, biofilter tank wall biofilm, rearing tank wall biofilm, as well as the treated water coming from the biofilter/upstream UV disinfection before returning to the rearing tanks. To the best of our knowledge, this is the first-time microbiota in both water and biofilm has been monitored with modern molecular methods over such a long timescale in a commercial RAS. The main objective of our study was to characterise and understand the spatial-temporal microbial community compositions and dynamics in both biofilm and water in the system, and to apply supervised machine learning demonstrating that microbiome profiles can be used for predictive and operational measures. We particularly aimed at documenting the dynamics of the general microbial community composition in contact with the salmon fry, the microbial community composition of the biofilter, and the effect of UV disinfection on the microbial population of the water in the RAS loop. This knowledge can contribute to improve the chemical and microbial water quality, to secure optimal production of Atlantic salmon in RAS for the future.

2. Materials and methods

2.1. Culture system and rearing regime

The study was based on samplings from a start-feeding department of a commercial RAS producing Atlantic salmon fry from 0.2 to around 3 g. The RAS facility was built in 2013 (Billund Aquaculture, Denmark) and is one of the largest producers of smolt in Norway. A total of seven production batches were cultivated in the monitored RAS during the period. Production batch 1 and 7 were only sampled for a part of the time the fish spent in the system. Between each production batch, there was a fallowing period for cleaning of rearing tanks with soap and hot water before a new group of fry was put in. The fallowing periods varied from 6 to 40 days, with an average of 24 days. During fallowing periods, the biofilters were fed 0.5 to 1 kg ammonium chloride (NH_4Cl) once a day, to maintain the nitrification activity. The ammonium chloride was added in the water sump before the biofilter (Fig. 1). The intake water from a lake (Heimsvatnet) was sand filtered and UV disinfected. The RAS consisted of six rearing tanks (dimensioned maximum biomass of 45 kg/m^3), with an associated water treatment loop consisting of a mechanical drum filter (60 μm mesh, Hydrotech, Veolia Water Technologies, Sweden) for particle removal, three fixed bed biofilters (FBBF) (3 × 13.5 m^3, RK BioElements, Denmark) for nitrification, a trickling filter (EXPO-NET BIO-BLOK®, 20 m^3, Denmark) for degassing of CO_2, and an ultraviolet irradiation treatment (MonoRay 10, UltraAqua, Denmark) of the full water flow for disinfection. The UV-dose was 35 mJ/cm^2. Also, the RAS included oxygenation from oxygen cones and pH regulation with calcium hydroxide slurry ($Ca(OH)_2$) added in the water sump before the biofilter, continuously and automatically. Make-up water was added in the water sump before the biofilter (Fig. 1). The system was socked with 2 kg/m^3 of Atlantic salmon fry and fed continuously with different commercial feeds of different pellet sizes (EWOS and Skretting, Norway). The three biofilters were backwashed with aeration every third week (one biofilter each week) to avoid clogging. The biofilters had never been disinfected throughout the seven years of operation. Final biomass at each production batch was between 14 and 45 kg/m^3. Total water flow in the start-feeding RAS was 454 m^3/h at all sampling times. The study resulted in 33 sampling timepoints (t0-t32). From t0 to t26 the rearing tanks had a water volume of 22.6 m^3, with a minimum hydraulic retention time (HRT) of 18 min. However, problems with removing particles from the rearing tanks resulted in a period of reconstruction from t27 to t29 (during fallowing) where all the rearing tank walls were extended with around 30–50 cm. After the reconstruction (t30-t32) the tanks had a volume of 35 m^3 and an HRT of 28 min. Production data and physicochemical water quality variables were provided by the RAS facility, including mortality, biomass of fish, feed type, temperature, total ammonia nitrogen (TAN), nitrite, nitrate, salinity, and pH.

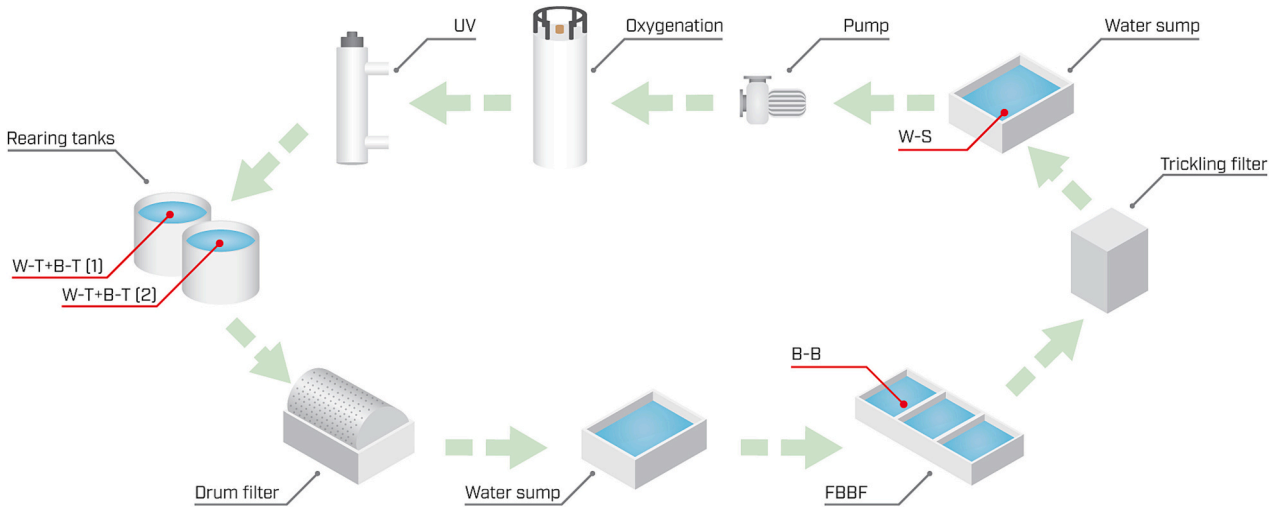

Fig. 1. Schematic presentation of the RAS monitored in this study. Sample points for RAS microbiota is presented as red lines: water samples from each of two rearing tanks (W-T), biofilm samples from the surface of walls of two rearing tanks (B-T), water from the sump downstream the biofilter and degasser and upstream the UV (W—S), and biofilm (B—B) from the fixed bed biofilter (FBBF). The UV disinfection was on full-flow. Illustration by Mats Mulelid, SINTEF Ocean. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.2. Sampling for microbiological analysis

Sampling for microbial community analyses was conducted biweekly over a 15-month period, from the 06th of November 2017 to the 28th of January 2019, resulting in 33 sampling timepoints (t0–t32) (Fig. 2). Samples were from four different points inside the RAS-loop: 1) water from two rearing tanks (W-T), 2) biofilm samples from tank walls (B-T) (same two rearing tanks as W-T), 3) biofilm samples from one of the fixed bed biofilters (B—B) and 4) water samples from a water sump (W—S) positioned after the biofilter, upstream the UV in the treatment loop (see Fig. 1). Water samples were collected by filtering 150–200 mL water through a 0.22 μm Sterivex filter (Millipore, USA) with Omnifix® syringes. Biofilm samples were taken by swabbing (Copan Diagnostics, USA) the tank walls of the two rearing tanks and inside the fixed bed biofilter. A new area was swabbed each time. All collected samples were stored in freezers (−20 °C at the facility, −80 °C at SINTEF) until further analyses were performed. A total of 244 samples were subjected to microbial community analysis by Illumina sequencing of 16S rDNA amplicons. Water samples were also collected for analyses of flow cytometry and colony forming units (CFU) at production day 30, 34 and 40 of production batch seven. Samples were taken from the same points as the water for microbial community analysis: the two rearing tanks and the water sump (Fig. 1).

2.3. Microbial community analyses

For DNA-extraction, two different kits were used: FastDNA® SPIN Kit for Soil (MP Biomedicals, USA) was used for samples taken from t0 to t17, while ZymoBIOMICS™ DNA Miniprep kit (Zymo Research, USA) was used for samples taken from t18 to t32. Extraction was done as described by the manufacturers. To check if there was a difference between the two extraction kits, DNA from the same samples was extracted with each kit. The extracted DNA was sequenced, and the microbial community composition results were subsequently compared at different taxonomical levels. Only small differences were found in the microbial community composition between the two DNA-extraction kits. The Genomic DNA Clean & Concentrator™-10 kit (Zymo Research, Irvine, California) was used to purify the DNA. The extracted DNA was sent to the Centre of Biotechnology (CeBiTec), Bielefeld University (Germany) for 16S rDNA amplicon library preparation and sequencing. Library preparation was conducted after standard Illumina instructions. The variable regions 3 and 4 (v3 + v4) of the 16S rRNA gene was amplified by two PCR rounds using the 2xHiFi HotStart ReadyMix (Kapa Biosystems, USA). To cover the domains of Bacteria and Archaea, the primers 341F (5'-CCTACGGGNGGCWGCAG-'3) and 805R (5′- GACTACHVGGGTATCTAATCC-3′) were used for the first PCR round (Takahashi et al., 2014). Obtained amplicons were indexed,

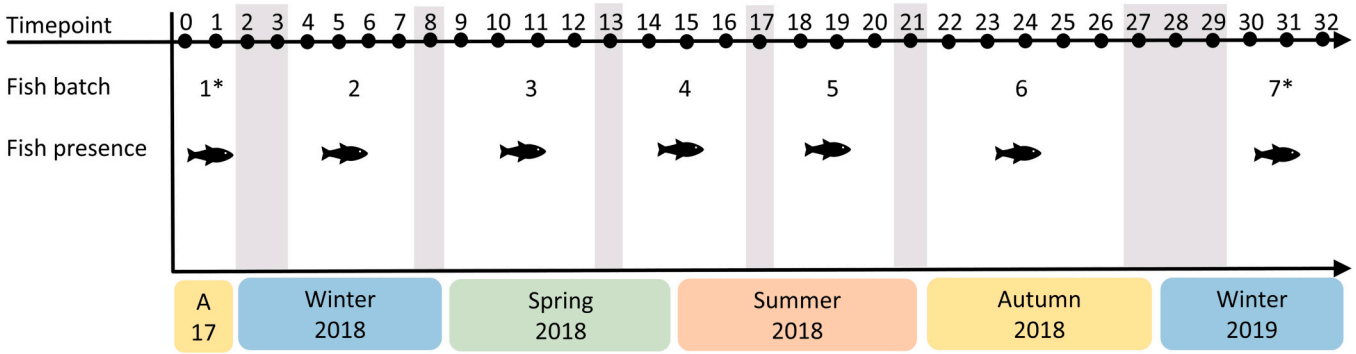

Fig. 2. Timeline for sampling for microbial community analyses during seven production batches. Sampling was conducted biweekly over a 15-months period, resulting in 33 sampling timepoints, from t0 to t32 (upper numbers). Shaded areas in between production batches represent the fallowing periods where there was no fish in the department. Production batch 1* and 7* were not followed for the whole production period, as batch 1 was only monitored the last 15 days and batch 7 the first 48 days of the batch period.

pooled and subsequently sequenced on an Illumina MiSeq platform (paired end sequencing; 2 × 300 bp). The Illumina sequencing data were processed with the USEARCH pipeline (version 9.2; https://www.drive5.com/usearch/). During merging of paired reads, primer sequences were removed and reads shorter than 380 base pairs were filtered out. The processing further included demultiplexing and quality trimming by the Fastq filter command (with an expected error threshold of 1). The UPARSE-OTU algorithm was applied for chimera removal and clustering at the 97% similarity level (Edgar, 2013). Taxonomy assignment was based on the SINTAX script (Edgar, 2016) with a confidence value threshold of 0.8 and the RDP reference data set (version 16). For identifying OTUs (Operational taxonomic units) potentially representing nitrifiers, the OTUs were also classified using the MiDAS 3.2 reference data set based on 16S rRNA gene sequences obtained from activated sludge wastewater treatment systems (Nierychlo et al., 2019). The resulting OTU table was normalised to 17 000 number of reads per sample by determining the fraction of the OTUs for each community profile, and subsequently multiplying by 17 000, and finally rounding off the read numbers to integers. A Maximum likelihood analysis was conducted to examine the phylogenetic relationships between the most abundant *Nitrospira* OTUs identified in this study and previously described *Nitrospira*, including representatives for comommox *Nitrospira*. 16S rRNA gene sequences were retrieved from the NCBI GenBank or the Ribosomal Database Project (RDP) database (Cole et al., 2014). The analysis was performed in MEGA-X software v. 10.2.4 (Kumar et al., 2018). The sequences were aligned using ClustalW with the default parameters. A maximum likelihood analysis was performed with 1 000 bootstrap replicates and the Tamura-Nei model for sequence evolution (Tamura and Nei, 1993). The resulting sequencing data are deposited at the European Nucleotide Archive (accession numbers ERS13478210-ERS13478454).

2.4. CFU analysis for estimating fraction of opportunistic bacteria

Agar plates was prepared by mixing 8.75 g PCA (plate count agar) (Himedia, India), 1.50 g agar powder and 500 mL Milli-Q water. Water samples were plated immediately after sampling. Samples were diluted and plated in triplicates on the petri dishes and incubated at 14 °C. Colony forming units (CFU) were registered after three and 18 days of incubation. Plates containing 30–300 colonies were used for counting. Opportunistic bacteria were defined as the fraction of CFUs registered three days after incubation of the total number of CFUs registered after 18 days of incubation (Skjermo et al., 1997).

2.5. Flow cytometry and growth potential

The total number of bacterial cells in water samples was determined by flow cytometry using a BD Accuri™ C6 Flow Cytometer (BD Biosciences, USA). Six replicates pr sample were fixated with glutaraldehyde (final concentration 0.01%) and stored in refrigerator for maximum three days prior to flow cytometry analysis. Samples were diluted 1:10 with TE buffer and further stained with a 1:50 working solution of SYBR® Green II RNA Gel Stain (Life Technologies, USA). After staining, samples were incubated in the dark for 15 min. A medium flow rate (35 μL min^{-1}) and a 4 min collection time was used for all samples for counting of bacterial cells. The FL1 detector was set to a threshold value of 3000. The gating that was used for all flow cytometry samples excluded fluorescent intensity signals below approximately 103.5 on the FL1 detector. Triplicate sub-samples from the same water sample were also incubated at 14 °C for three days in open 50 mL plastic tubes to determine the bacterial growth potential. After three days, samples were subjected to flow cytometry as described above. The bacterial growth potential was calculated as the fraction of total bacteria after three days incubation compared to the original number of total bacterial cells (Attramadal et al., 2016).

2.6. Supervised machine learning

The variations in microbial community composition in the biofilter biofilm and the water samples (rearing water and water sump) were examined further by using supervised machine learning (SML) models. The aim was to examine the power of measured physicochemical water quality variables and other production parameters for prediction of the total microbial community profile dynamics. The variables that were processed included: temperature, salinity, oxygen saturation, pH, nitrogen waste products (TAN, NO_2^-, NO_3^-), mortality, fish presence, biomass of fish (kg/m^3), and feed type (Ewos start and Skretting Nutra Sprint). SML algorithms aim at extracting information from a training dataset into a predictive model that has a potential to class labels on upcoming, unlabelled samples (Cordier et al., 2019). In this context, obtained OTU table was used as the input dataset (features), while metadata file containing physicochemical and production parameters was used as endpoint information. The total dataset was split into a training dataset and a model evaluation dataset, contributing to 75% and 25% of total number of samples, respectively. Random forest machine learning algorithm was applied to the data, through Quantitative Insight into Microbial Ecology 2 (qiime2) pipeline v.2021.2 (Boylen et al., 2019) based on scikit-learn python machine learning package v.0.23.1. Both numerical and categorical type of predictors were used, based on the parameters used.

2.7. Statistical analyses

The USEARCH commands Alpha_div and Sintax_summary was used to calculate alpha diversity indices (observed OTU richness and Shannon's diversity) and generate taxa summary tables, respectively. PAST (version 4.0; Hammer et al., 2001) was used to calculate Bray-Curtis similarities. Principal Coordinate Analysis (PCoA) ordinations based on Bray-Curtis similarities (Bray and Curtis, 1957) were made to illustrate the beta-diversity (Hammer et al., 2001). One-way PERMANOVA (permutational multivariate analysis of variance) based on Bray-Curtis similarities were used to test if there was a statistically significant difference between sample-groups (Anderson, 2001), with the significance threshold set to a *p*-value below 0.05. When more than two groups were compared, one-way PERMANOVAs with Bonferroni-corrected *p*-values were used. SIMPER (Similarity Percentage) analysis based on Bray-Curtis values was performed to identify the OTUs which contributed the most to the difference in microbial community composition between selected groups (Clarke, 1993). Standard error (SE) was used to show the variation of data.

3. Results

3.1. Physicochemical water quality

The physicochemical water quality variables were generally satisfying for salmon production and relatively similar among the production batches examined. The salinity was raised occasionally when the RAS-facility encountered problems with water mold, resulting in a variation in salinity from 0.3 to 2.5 ppt during the period (Table 1). The oxygen saturation never fell below 91.0% and the pH was stable, varying between 6.9 and 7.0. The concentrations of total ammonia nitrogen (TAN), nitrite (NO_2^-) and nitrate (NO_3^-) tended to increase throughout the production batches, as expected (Fig. 3; Fig. S1, Supplementary). There were fluctuations in both NO_2^- and NO_3^- concentrations during the period, varying between 0.05 and 0.6 mg/L and 78–194 mg/L, respectively. It was visually observed more particles in the water in production batch 6 (Fig. S1, Supplementary), compared to the other batches. During fallowing periods, temperature, salinity, and the concentrations of nitrogen products were lowered, while oxygen saturation increased, as expected. The pH did not change during fallowing.

Table 1
Physicochemical water quality for the seven production batches and the six fallowing periods (average ± SE). All variables were measured in the rearing tanks (Fig. 1), except from pH, which was measured in the water sump after the biofilter. Fall = fallowing, TAN = total ammonia nitrogen. Data are missing from Fall 6 due to reconstruction of rearing tanks and no measurements.

	Temperature (°C)	Oxygen saturation (%)	pH	Salinity (ppt)	TAN (mg TAN/L)	Nitrite (mg NO_2^-/L)	Nitrate (mg NO_3^-/L)
Batch 1	12.9 ± 0.7	91.0 ± 0.9	6.9	2.0 ± 0.0	1.1 ± 0.2	0.6 ± 0.1	177.1 ± 7.5
Fall 1	12.1 ± 0.1	100.2 ± 0.4	6.9	1.1 ± 0.0	0.2 ± 0.0	<0.05	85.8 ± 8.8
Batch 2	13.8 ± 0.1	92.2 ± 0.3	7.0	2.5 ± 0.2	0.6 ± 0.2	0.3 ± 0.1	113.9 ± 13.0
Fall 2	12.0 ± 0.2	104.0 ± 0.4	7.0	1.0 ± 0.1	0.5 ± 0.1	<0.05	78.0 ± 9.5
Batch 3	13.7 ± 0.1	93.7 ± 0.9	6.9	1.9 ± 0.2	0.7 ± 0.1	0.2 ± 0.1	143.8 ± 22.2
Fall 3	12.3 ± 0.1	101.5 ± 0.9	6.9	1.0 ± 0.0	0.6	<0.05	93.0 ± 0.0
Batch 4	13.9 ± 0.1	92.8 ± 0.3	6.9	2.5 ± 0.1	0.5 ± 0.0	0.2 ± 0.0	179.7 ± 26.6
Fall 4	13.5 ± 0.3	99.4 ± 0.4	6.9	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	97.0 ± 33.0
Batch 5	14.0 ± 0.1	93.2 ± 0.2	6.9	0.9 ± 0.0	0.5 ± 0.0	0.1 ± 0.0	120.9 ± 17.9
Fall 5	13.8 ± 0.1	99.7 ± 0.3	6.9	0.7 ± 0.1	0.2 ± 0.0	<0.05	140.0 ± 10.0
Batch 6	13.7 ± 0.1	92.5 ± 0.5	7.0	1.1 ± 0.0	0.8 ± 0.1	0.3 ± 0.1	194.6 ± 21.5
Fall 6	–	–	7.0	–	–	–	–
Batch 7	13.5 ± 0.1	92.3 ± 0.2	6.9	1.6 ± 0.1	0.7 ± 0.1	0.2 ± 0.1	135.7 ± 14.0

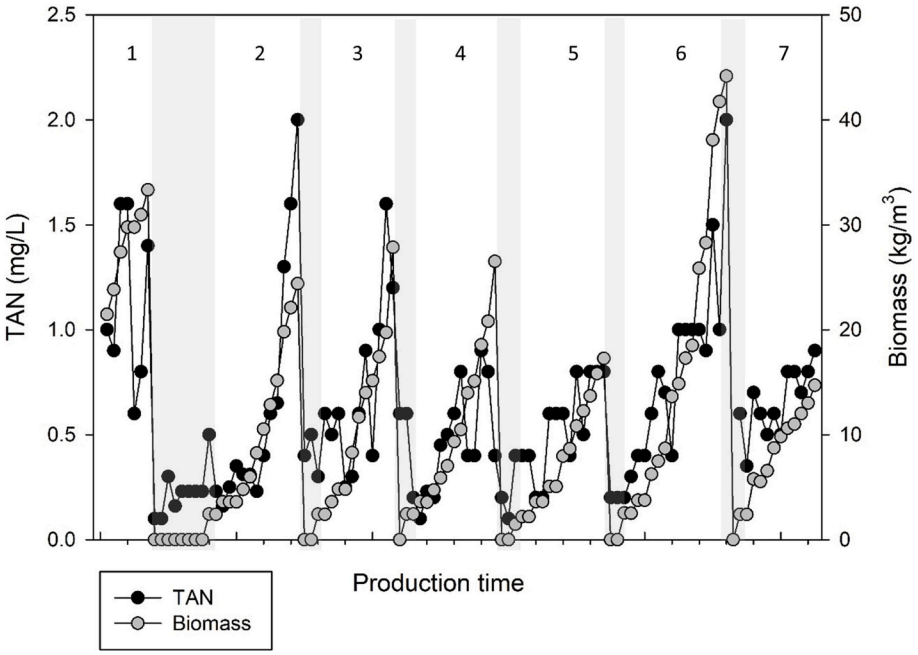

Fig. 3. Total ammonia nitrogen (TAN) concentration and biomass of fish (kg/m^3) during the seven production batches (upper number). Shaded areas represent the fallowing periods. TAN was measured in rearing tanks (Fig. 1). The suggested threshold for TAN in Norwegian aquaculture producing Atlantic salmon in freshwater is <2 mg/L (Hjeltnes et al., 2012).

3.2. Fish performance

The average daily mortality was 0.11 ± 0.01% during the 15 months for all production batches. During a production batch, the daily mortality usually peaked during day 2–3 after inset of fish, after which it stabilized and decreased towards the end of the production period (Fig. 4). The exception was production batches 2 and 3, which also had an increase in daily mortality in the middle of the production period. The two rearing tanks examined in the study had approximately the same pattern of daily mortality (0.10 ± 0.01%; 0.12 ± 0.01%, respectively) throughout the period (Fig. 4). The daily mortality was significantly different between the production batches (Kruskal-Wallis, p = 0.001), where production batch 7 had the highest single incident of mortality in both rearing tanks on day 13 and was the production batch with the highest average daily mortality (0.21 ± 0.06%) (Fig. 4, Table S1, Supplementary). Production batch 5 had the lowest average daily mortality (0.06 ± 0.01%), for the completed batches (batch 2–6) (Table S1, Supplementary).

The average final fish weight was similar between the batches, with 2.69 ± 0.21 g, except production batch 6 with an average of 3.90 g final weight. In this batch, the fish was kept in the RAS for a longer period (Table S1, Supplementary). Also, the specific growth rate (SGR) was similar, ranging from 5.15 to 5.29% for the completed batches (Table S1, Supplementary).

3.3. Microbial community composition and dynamics in the RAS

3.3.1. Composition of the water and biofilm microbiota

Ordination by Principal Coordinate Analysis (PCoA) indicated differences between microbial community structures in water (rearing water, water sump downstream the biofilter/upstream the UV disinfection) and biofilm (biofilter and tank wall) samples, and biofilm from biofilter and tank wall (Fig. 5). Significance of observed differences was confirmed by one-way PERMANOVA test ($p = 1.0 \times 10^{-4}$).

Despite significant differences in community compositions in general, the water samples from the two rearing tanks and the water sump

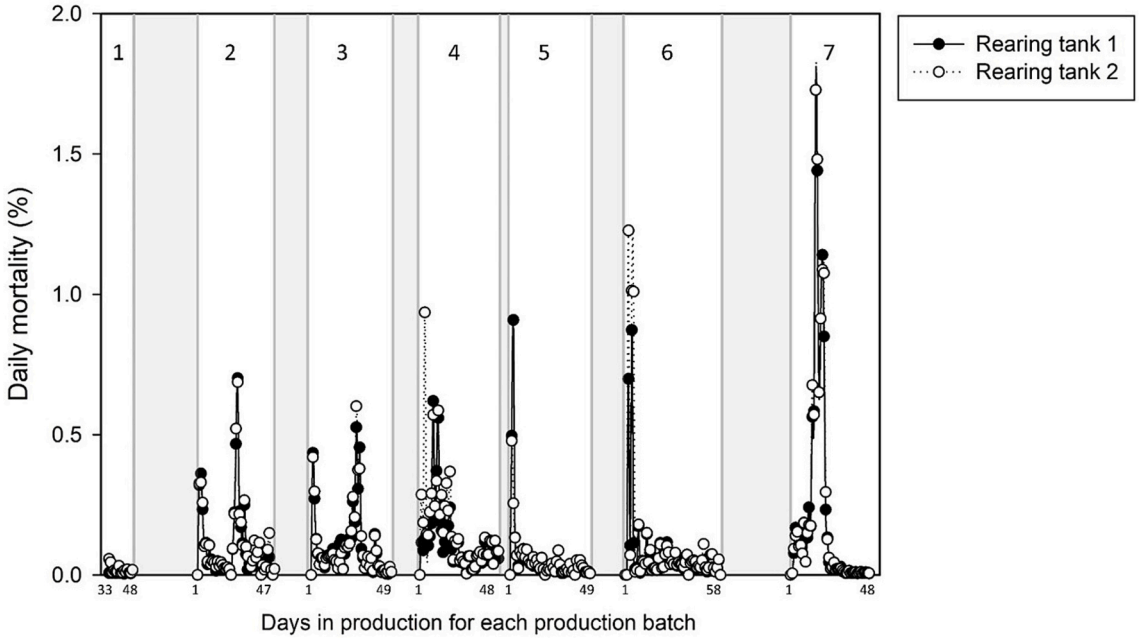

Fig. 4. Daily mortality (%) during the production period for the two rearing tanks trough seven different production batches (upper numbers, 1–7). Shaded areas represent fallowing periods and the numbers on the x-axis represent the day in production for each batch (from day 1 up to 58 days).

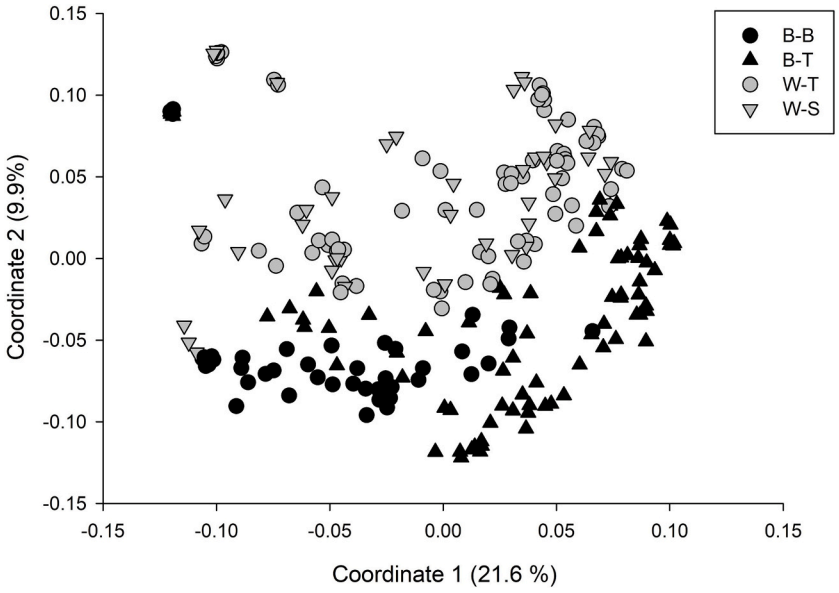

Fig. 5. PCoA ordination based on Bray-Curtis similarities for water (grey symbols) and biofilm (black symbols) samples. Water samples included water from the two rearing tanks (W-T) and the water sump downstream the biofilter/upstream the UV disinfection (W—S), biofilm samples from tanks walls (B-T) and the biofilter (B—B) over a period of 15 months, total 33 timepoints. Triplicates were included from timepoint 0 to 5. n = 43 (B—B), n = 76 (W-T), n = 43 (W—S), and n = 81 (B-T).

were similar in composition (PERMANOVA, $p > 0.24$). The biofilm and water samples showed variation in composition over time, both within and between the production batches (Fig. 6A, B).

Alpha diversity expressed as exponential Shannon's index showed that the biofilter biofilm (B—B) had a significant higher diversity than the other sample groups (Fig. 7) (ANOVA, $p < 0.05$) except the water sump (W—S). The biofilm on the tank walls had a significant lower diversity, both in terms of OTU richness and exponential Shannon's index than the other sample groups (ANOVA, $p < 0.05$). The water from the water sump located after the biofilter/before the UV (W—S) had both higher richness and exponential Shannon's diversity compared to the rearing tanks (W-T), although not significant.

The most abundant orders in the biofilter biofilm communities were Rhodobacterales (average 9.4 ± 1.4%), Thiothrichales (8.5 ± 2.6%), Rhizobiales (7.8 ± 0.6%), and Burkholderiales (5.9 ± 0.8%) (Fig. 8A). The nitrite-oxidising order Nitrospirales was the 8th most common order with an average relative abundance of 3.8 ± 2.8%. The ammonia oxidising Nitrosomonadales, on the other hand, had an average relative abundance of only 1.0 ± 0.9% (Fig. 8A). For the biofilm on the tank wall of the two rearing tanks, the most common order was Rhodobacterales (21.7 ± 2.7%), Burkholderiales (average 17.4 ± 2.2%), Flavobacteriales (7.4 ± 1.5%), and Sphingomonadales (7.1 ± 1.3%) (Fig. 8B). The most common orders in the water microbiota were Burkholderiales (average 17.4 ± 1.3%) and Rhodobacteriales (8.5 ± 1.1%), which were also were included in the top four orders for biofilter biofilm and biofilm tank wall. Further, Sphingomonadales (6.6 ± 0.7%) and Chlamydiales (6.3 ± 0.9%) (Fig. 8C) were abundant taxa in water samples (Fig. 8C).

To identify which OTUs contributed most to the difference between all samples of water and biofilm, a SIMPER analysis based on Bray-Curtis similarities was conducted. Collectively, ten OTUs contributed with

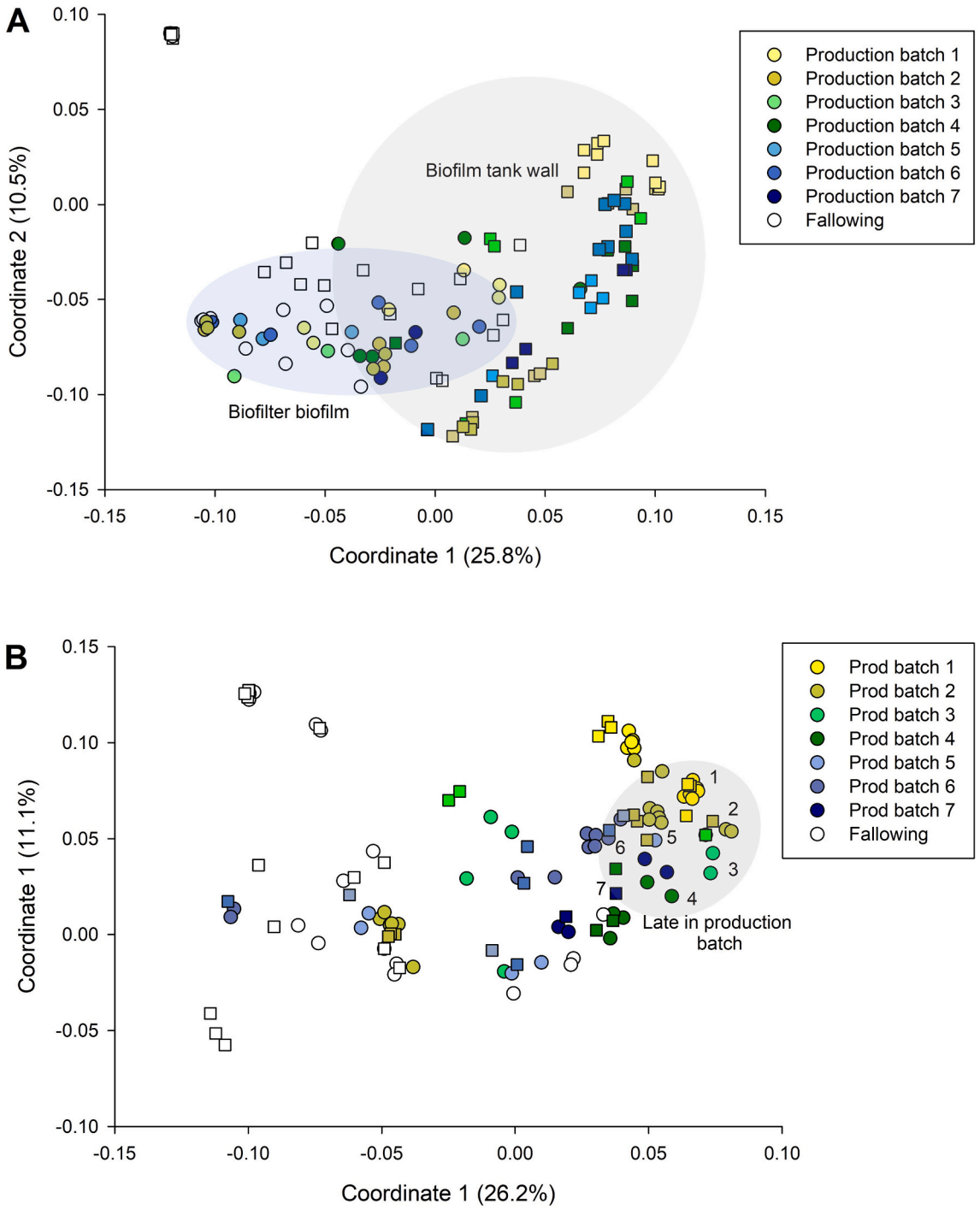

Fig. 6. PCoA-plot based on Bray–Curtis similarities sorted by the seven different batches of fish and the six fallowing periods for A) biofilm samples from the biofilter and the tank walls through the seven batches of fish and the fallowing periods. Circles = Biofilter biofilm, Square = tank wall biofilm, and B) water samples from two rearing tanks and the water sump. Circles = rearing tanks, Square = water sump. Samplings late in production batch symbolised by numbers of a given batch and a shaded area. n = 43 (Biofilter biofilm), n = 81 (Biofilm tank wall), n = 76 (rearing tanks), n = 43 (water sump).

nearly 30% of the differences between the samples (Table 2). OTU_1, representing the family Rhodobacteraceae, was the most contributing OTU, singularly explaining almost 10% of the differences. OTU_1 was far more abundant in the water and in the biofilm on tank wall (16.50; 17.70%) compared to the biofilter biofilm (6.49%). This observation was also reflected in the taxa plot (Fig. 8), where Rhodobacterales was highly more abundant in tank wall biofilm (21.69%) compared to the other sample groups (8.46–9.43%), reaching maximum abundance of 51.71% at timepoint 12. OTU_2 assigned as *Thiothrix* was the second most contributing OTU, with a higher relative abundance in the biofilter biofilm (7.28%) compared to the other locations (1.50–1.87%). This OTU was dominating the order Thiotrichales which was far more abundant in the biofilter biofilm (8.51%), compared to the other sample groups (1.81–2.42%) (Fig. 8). OTU_4 (Burkholderiales inceartae sedis, *Sphaerotilus*) was hardly present in the rearing water or biofilm tank walls but present in biofilm biofilter (1.86%) and the water sump (6.67%). On order level however, Burkholderiales was abundant in similar levels in tank water and tank biofilm (17.40%) while the biofilter

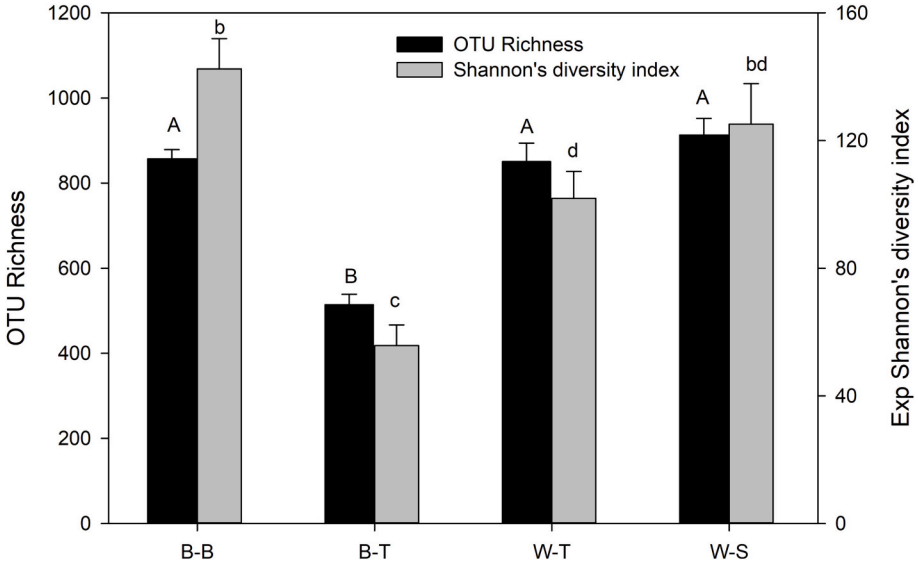

Fig. 7. Alpha diversity indices expressed as the average observed OTU richness and exponential Shannon's diversity index ($e^{Shannon}$). B-B = biofilter biofilm, B-T = tank wall biofilm, W-T = water rearing tanks, W-S = water sump downstream the biofilter and upstream the UV. The indices were calculated as the mean (±SE) of all sampling times (t0-t32). $N = 43$ (B—B), n = 76 (W-T), n = 43 (W—S), n = 81 (B-T). Different letters indicate significant differences for OTU richness (capital letters) and exponential Shannon's diversity (lower-case letters).

biofilm revealed some lower abundance (5.92%). Pseudomonadales were among the top ten most abundant taxa in both biofilm types but were not detected in water. Taxa found to be abundant in biofilter biofilm and water, but not in tank wall biofilm included Nitrospirales and Caldilineales, where biofilter biofilm had the highest abundance of Nitrospirales, as expected. Chlamydiales was typically more abundant in water than biofilms.

3.3.2. *Temporal dynamics of water and biofilm communities*

Both biofilter biofilm, tank wall biofilm and water showed variation in the microbial communities over time (Figs. 6, 7, 8). Bray-Curtis similarities for the whole monitored period showed that the highest variation in microbial communities was observed for the tank wall biofilm ($0.30 \pm 3.0 \times 10^{-3}$). The rearing water ($0.35 \pm 2.0 \times 10^{-3}$) and water sump ($0.36 \pm 2.0 \times 10^{-3}$) had similar variation, and biofilter biofilm the lowest variability over time (0.35 ± 0.01). A common feature was that all samples, both biofilm and water, clustered according to production batches and fallowing periods in the PCoA ordination (Fig. 6) and differed significantly between these two states (one-way PERMANOVA, water $p = 1.0 \times 10^{-4}$; biofilter biofilm $p = 2.0 \times 10^{-4}$; tank wall biofilm $p = 1.0 \times 10^{-4}$). This was also reflected in a moving window analysis, comparing the community composition at subsequent sampling times, where the lowest Bray-Curtis similarities were at the fallowing periods (Fig. 9). The fallowing periods seemed thus to affect the microbiota substantially. One of the most striking differences in microbial communities between fallowing and production periods was a strong increase in abundance of Rhodobacterales during production periods, especially for the tank wall biofilm and water (Fig. 8B, C). The microbial communities changed during fallowing, but the microbiota was developing back to the composition that was present before the fallowing, during the production batches. This was evident in the PCoA-plot where the samples from late in each production batch clustered together and was particularly evident for the water samples (Fig. 6B).

Although the community composition of the biofilm on the tank walls and in the biofilter and the water were significantly different, the samples from all locations generally followed the same temporal pattern in similarity as shown in the moving window analysis (Fig. 9). The biofilter biofilm community composition was surprisingly varying over time (Figs. 6, 8, 9). The abundance of Thiothrichales showed large variations in relative abundance over the 15 months; it increased during production batch 4 (up to 43.8%) and the subsequent fallowing period, accounting for as much as 60.0% of the total reads at the subsequent fallowing (t17) (Fig. 8A). At this timepoint Thiothrichales were dominated completely by only one OTU classified as *Thiothrix* (OTU_2). For the same production batch, *Thiothrix* also increased in relative abundance in tank wall biofilm (42.30%). Rhodobacteriales and Pseudomonadales were also predominant orders that varied highly in abundance during the monitored period. The water microbiota changed the most during the three first fallowing periods (Figs. 8, 9). At these fallowing periods the abundances of Burkholderiales decreased and Chlamydiales and Sphingomonadales increased. Chlamydiales reached maximum abundance of 37% in production batch 3, compared to 8% in the last batches (Fig. 9). Both the PCoA-plot, moving window analysis and Bray-Curtis similarities showed that the water microbiota was generally similar between samples taken from the two rearing tanks and the water sump (Fig. 8C, PERMANOVA $p = 1.0$) with Bray-Curtis similarity of 0.82 ± 0.03 during the period (Fig. S3, Supplementary).

3.3.3. *Nitrifying communities in the biofilter*

OTUs potentially representing nitrifying bacteria were identified by manual inspection of the OTU table. We identified four OTUs representing the nitrite-oxidising (NOB) genus *Nitrospira*, and five OTUs represented the ammonia-oxidising (AOB) genus *Nitrosomonas* or the family Nitrosomonadaceae (Fig. 10). The four *Nitrospira* OTUs accounted for in average 77% of the total reads for the OTUs classified as nitrifiers, while the five *Nitrosomonas*/Nitrosomonadaceae OTUs comprised only on average 23%. The total abundance of the OTUs representing nitrifiers accounted for a relatively low proportion of the total reads in the samples, with maximum abundance of 12.5% (Fig. 10). Their relative abundances varied both within and between production batches. Production batch 4 had considerably lower abundance of nitrifying OTUs, than the other production batches (average of 1.1%) and the subsequent fallowing period (0.54%). The relative abundance of nitrifiers tended to increase at the fallowing periods or immediately after the fallowing and to decrease throughout the production batches (Fig. 10). The low AOB:NOB ratio (average 0.37) for OTUs representing NOBs and AOBs could potentially be explained by some of the *Nitrospira* OTUs representing complete ammonia oxidisers (comammox). We therefore performed a phylogenetic analysis to investigate the relationships between the *Nitrospira* OTUs identified here and previously described *Nitrospira*, including both NOB and comammox *Nitrospira* members. Interestingly, maximum likelihood analysis indicated that the *Nitrospira* OTU_1771 was closely related to the comammox *Nitrospira nitrificans* (Fig. 11). OTU_1771 was on average the third most abundant OTU of all *Nitrospira* in the biofilter biofilm with an average relative abundance of 0.85% and maximum relative abundance of 2.96%.

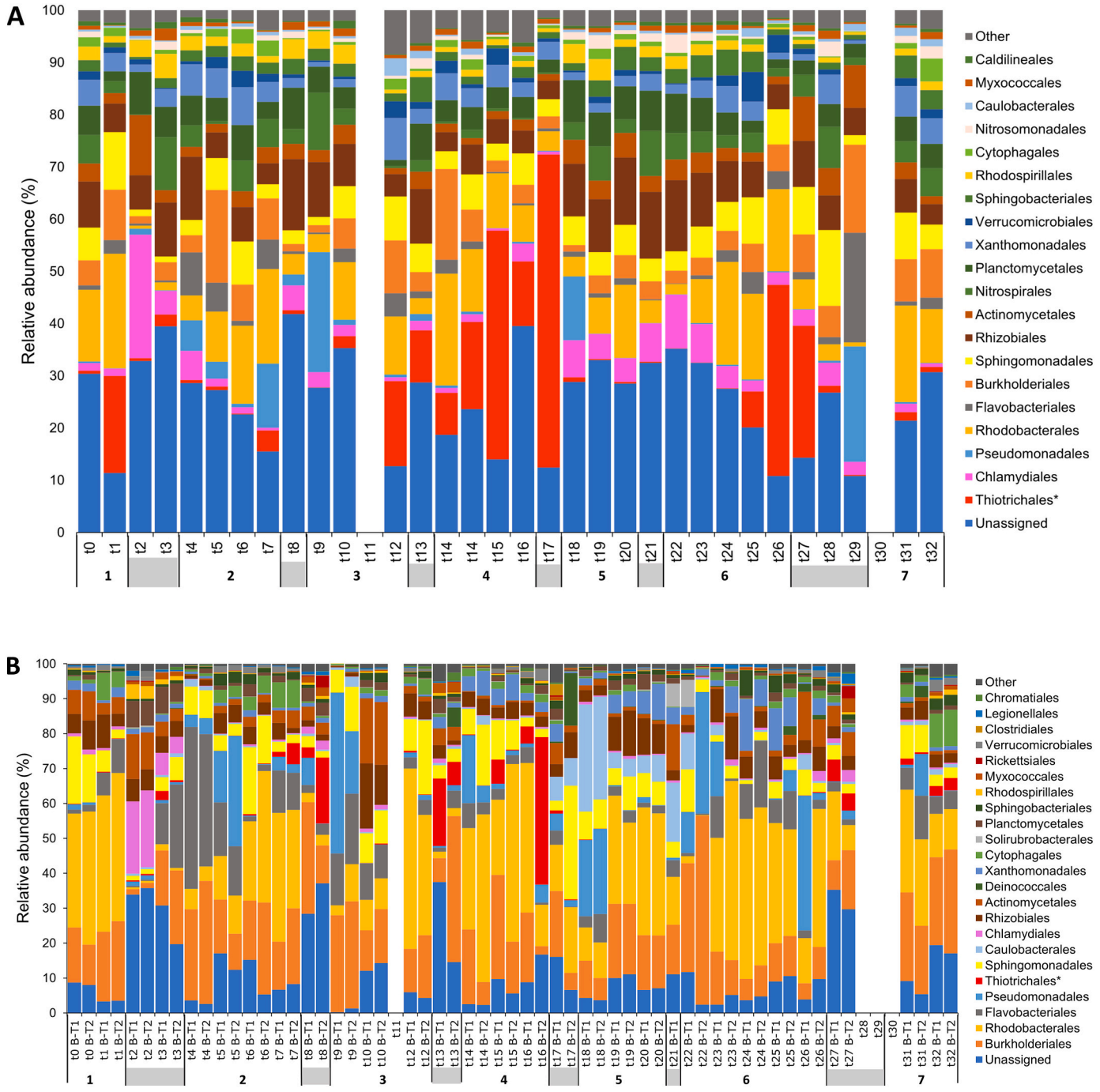

Fig. 8. Microbial community composition at order level for A) biofilm samples from the fixed bed biofilter, B) biofilm samples from the tank wall of the two rearing tanks (B-T1 and B-T2) and C) water samples from the two rearing tanks (W-T1 and W-T2) and the water sump (W—S) for sampling time t0-t32. Orders with relative maximum abundance below 2% in all samples are included in "other". The numbers below the x-axis represent the seven production batches, with fallowing periods in shaded areas between. *Included in Thiothrichales is OTU_2 that was manually classified as *Thiothrix* using the RDP Classifier.

3.3.4. *Factors affecting the microbial communities in RAS*

We used supervised machine learning (SML) to investigate correlations between the composition of microbial communities in the water (both rearing water and water sump) and the biofilter biofilm with the measured physicochemical water quality and other rearing production parameters. Community composition in rearing tanks and water sump exhibited excellent predictability towards fish presence (100%), and good predictability towards biomass (83%) and oxygen saturation (85–88%) (Table 3). In addition, microbial community of rearing tanks showed to be an excellent predictor of feed type used during the production (93%). Microbial communities of biofilter biofilm showed to predict only the fish presence (89%), among all the parameters tested. Lastly, mortality, salinity, pH and nitrogenous compounds (TAN, NO_2^-, NO_3^-) showed poor predictability based on microbial community dynamics of all sample types (below 80%). Finally, we examined which OTUs contribute the most to the predictability strength of sample types and parameters that display good and excellent predictions. The OTUs and corresponding taxonomy can be seen in Fig. S3-S9 (Supplementary).

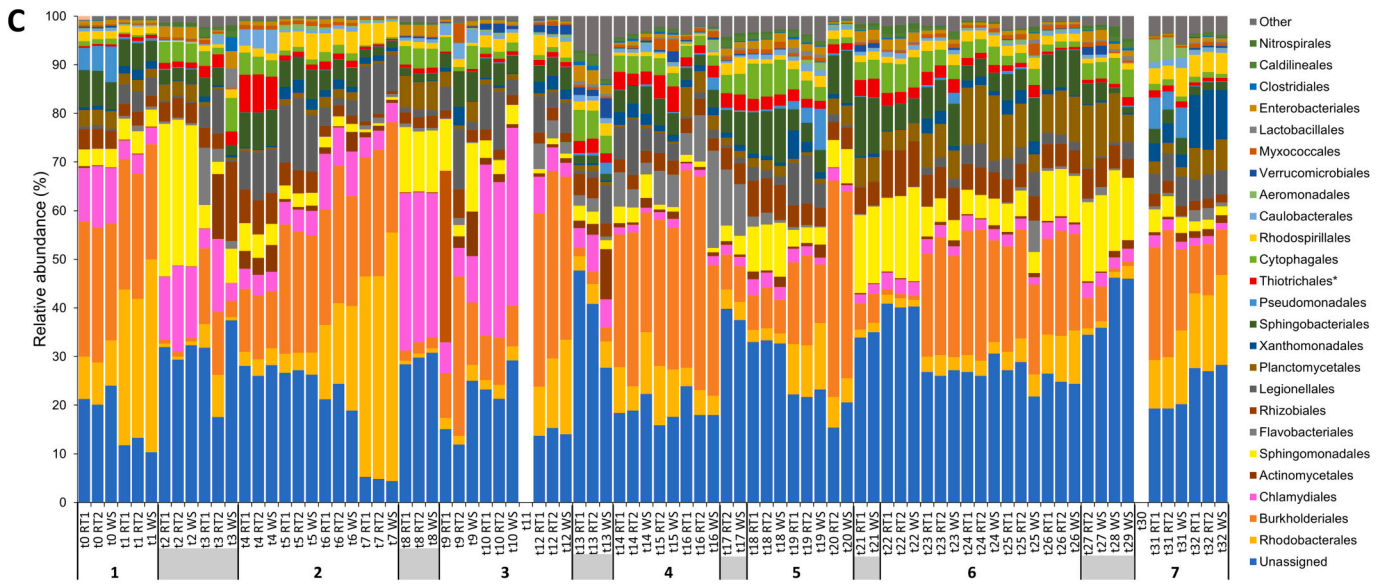

Fig. 8. (*continued*).

Table 2
The ten OTUs contributing most to the difference between the microbial communities in biofilter biofilm (B—B), tank wall biofilm (B-T), rearing water (W-T) and water sump (W—S), identified by SIMPER-analysis based on Bray-Curtis similarities. The relative abundances are specified as percentages of the total reads and represent averages between all samples in the relevant sample group.

			Relative abundance (%)			
OTU	Taxonomy	Contribution (%)	B-B	B-T	W-T	W-S
OUT_1	f:Rhodobacteraceae	9.73	6.49	16.50	13.05	17.70
OUT_2	f:Thiotrichaceae, g:*Thiotrix**	3.31	7.28	1.60	1.52	1.87
OUT_4	f: Burkholderiales_incertae_sedis, g:*Sphaerotilus*	2.97	1.86	0.44	0.22	6.67
OUT_9	f:Comamonadaceae, g:*Rhodoferax**	2.80	1.41	3.49	3.98	2.48
OUT_3	f:Mycobacteriaceae, g:*Mycobacterium*	2.51	1.09	3.57	3.17	1.52
OUT_17	f:Sphingomonadaceae	1.51	1.08	1.44	1.66	2.45
OUT_5	o:Actinomycetales*	1.43	0.71	2.11	0.83	2.09
OUT_11	f:Parachlamydiaceae	1.43	1.83	1.73	2.15	0.60
OUT_12	f:Flavobacteriaceae, g:*Chryseobacterium*	1.36	0.17	1.66	0.83	1.84
OUT_8	f:Moraxellaceae, g:*Acinetobacter*	1.27	1.03	0.23	0.06	2.21

* OTU_2, OTU_5, and OTU_9 was classified subsequent to the Usearch data processing using the RDP Classifier tool. The taxonomy for the OTUs is given at the lowest level obtained in the classification, either at order- (o), family- (f) or genus- (g) level.

3.4. Culturable bacteria and total bacterial cell numbers in the water samples

For the last production batch, analysis of culturable bacteria (colony forming units (CFUs)) and total bacterial cell densities (flow cytometry) were included, on three different production days. On production day 30, the fraction of fast-growing, potentially opportunistic bacteria in the rearing tanks were significantly higher than in the rearing tanks compared to the water sump downstream the biofilter (*t*-test, $p < 0.001$) (Fig. 12). On day 34, there was no significant difference, while on day 40 there was a higher fraction of fast-growing bacteria in the rearing tanks, compared to the water sump, although not statistically significant (Fig. 12). The rearing tanks had higher total bacterial cell densities than the water sump, although not significantly different (Table 4). The microbial growth potential was estimated by calculating the fraction of total bacteria after three days incubated on agar compared to the original number of total bacterial cells. The growth potential was lower in the water sump downstream from the biofilter compared to the water from the rearing tanks, although not significant. Altogether, the analyses of culturable bacteria indicated that there was a tendency of higher growth of presumptive opportunistic bacteria in the rearing tanks compared to the treated water downstream of the biofilter.

4. Discussion

This study aimed to characterise and understand the temporal dynamics of the complex microbial communities in a commercial RAS, during start-feeding of Atlantic salmon fry. To the best of our knowledge, this is the first-time microbiota of both water and biofilm has been regularly monitored over such a long timescale (15 months) in a commercial facility. The fish were healthy throughout the sampling period, and the results represent normal conditions for fry production in the studied RAS. Fish growth (SGR 5.2%) and daily mortality (0.11%) was normal during the monitored period. The physicochemical water quality variables were, in the context of commercial production, satisfying and relatively stable during the monitored period, indicating a well dimensioned RAS. However, the biomass was high at the end of production batch 6 (45.70 kg/m^3) (Table S1, Supplementary) which probably was the reason for more particles in the rearing tanks in this production batch. The biofilter was however seemingly effective and no increased mortality was observed.

Microbial communities in water, biofilm from rearing tanks and biofilter were all significantly different from each other. The most apparent difference on OTU level was OTU_1 (Rhodobacteraceae) that was far more abundant in the tank (water and biofilm) compared to the biofilter biofilm. Our results are in accordance with a study by Rud et al.

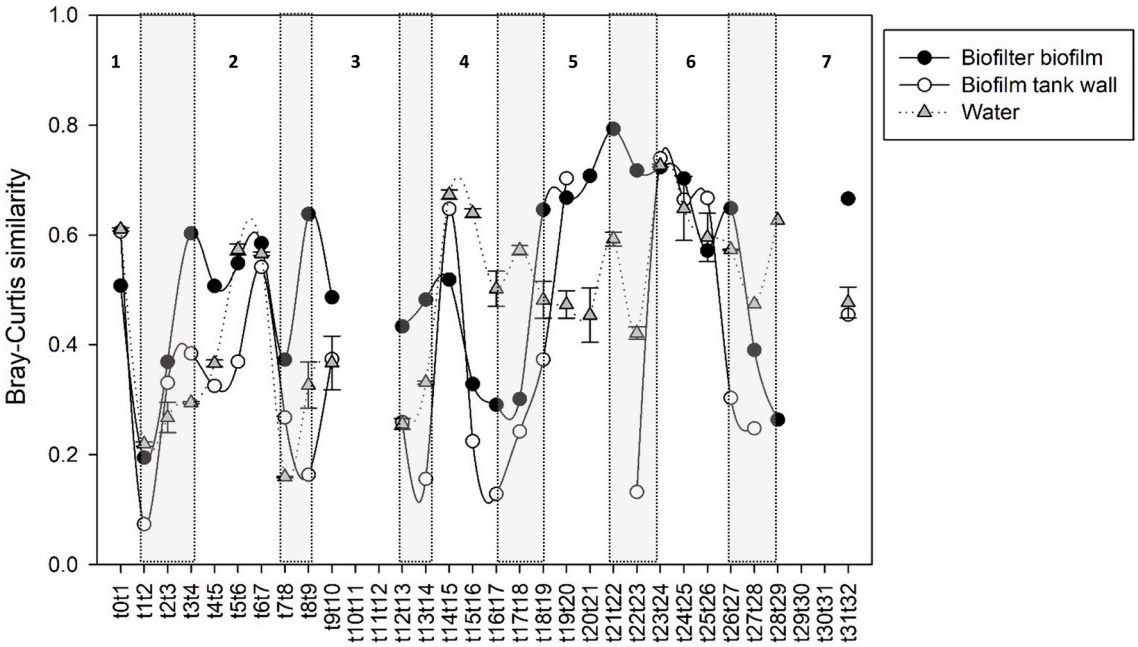

Fig. 9. Moving window analysis for comparing microbial community composition of one sampling time to the following sampling time, based on average Bray-Curtis similarity for biofilter biofilm, tank wall biofilm and average of the water (the two rearing tanks and the water sump). Upper numbers represent the seven production batches and shaded timepoints the fallowing periods. Circles represent biofilm, triangle water. Error bars for water represent average SE for water in the rearing tanks and sump.

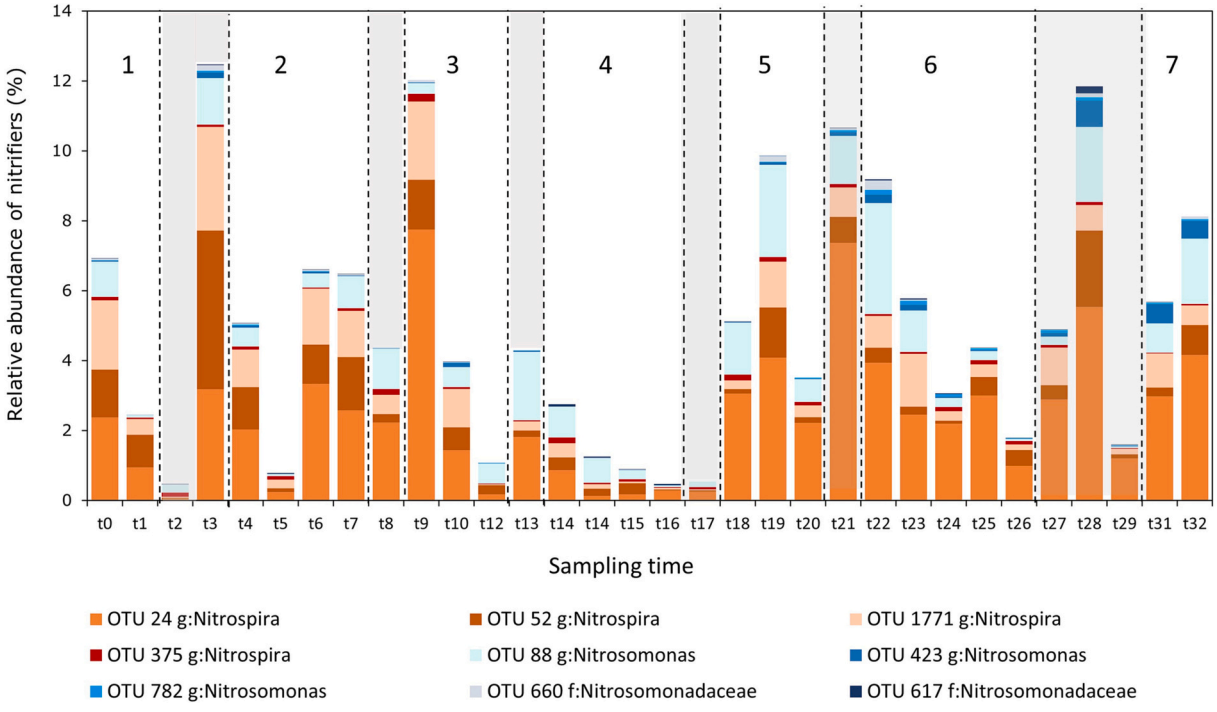

Fig. 10. Relative abundance of OTUs classified as nitrifying bacteria in the biofilter biofilm samples (t0-t32) for 15 months period. The taxonomy of the OTUs is given on the lowest obtained taxonomic level, genus (g) or family (f), classified by using the Usearch Sintax script and the RDP training set v18 or MiDAS. Three replicates are included in sampling times t0–5. The upper numbers represent the seven production batches, with fallowing periods in grey shaded areas between. Red bars = *Nitrospira*, blue/green bars = *Nitrosomonas*/Nitrosomonadaceae. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(2017) where Rhodobacteraceae was far more abundant in water compared to the biofilter biofilm. Rhodobacteraceae are well known for their metabolic versatility which contribute to nutrient cycling (Duarte et al., 2019). The second most contributing OTU, represented by *Thiothrix*, was more abundant in the biofilter biofilm than in the samples from tank (water and biofilm). *Thiothrix* have been identified previously in RAS, but at lower abundances (Rurangwa and Verdegem, 2014; Rud et al., 2017) and are capable of autotrophic denitrification (Rurangwa

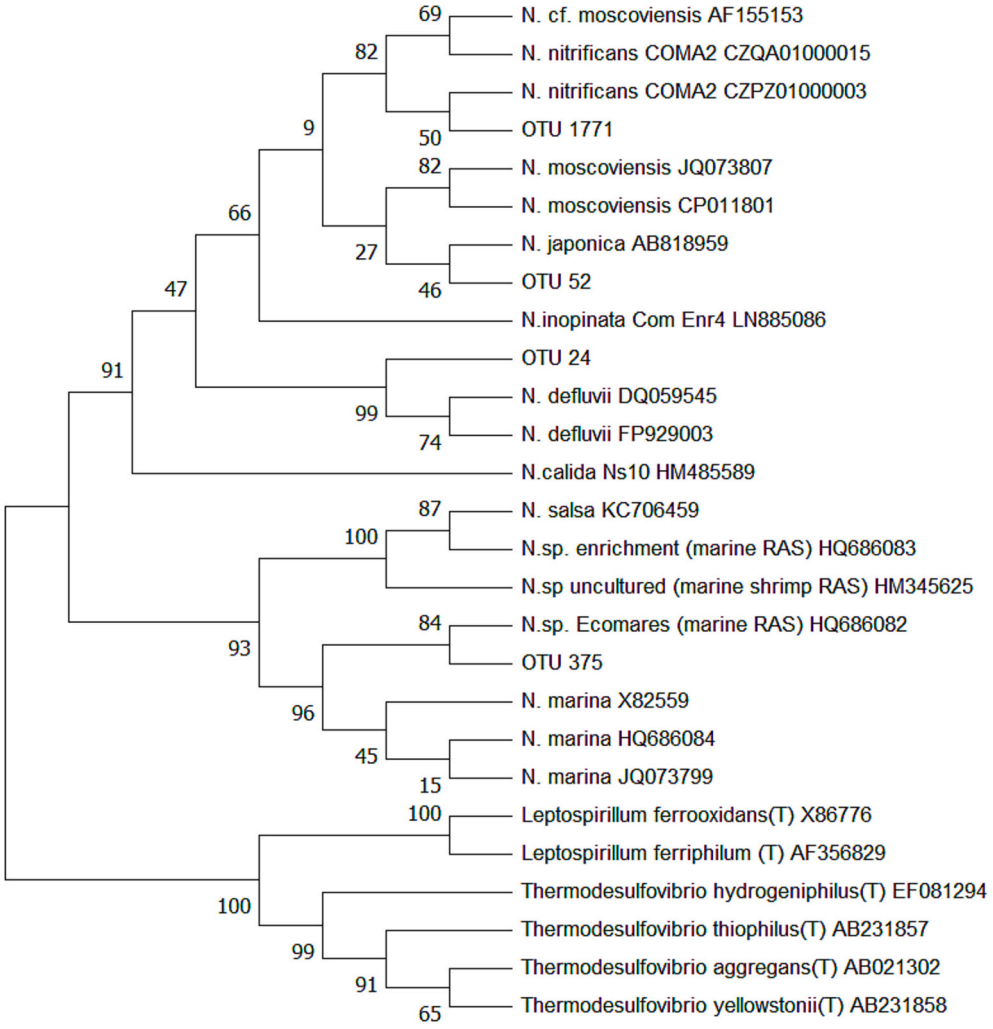

Fig. 11. Maximum likelihood (ML) tree for comparing OTUs classified as *Nitrospira* to previously published *Nitrospira* 16S rRNA gene sequences. Sequences were retrieved from RDP (Cole et al., 2014) or the NCBI Genbank. Accession numbers are specified for with the species names. Sequences representing comammox candidates are denotated "comammox". Type strains are indicated by a (T). The ML analysis was performed with 1000 bootstrap replicates and the Tamura-Nei model for sequence evolution. The three was condensed with 50% cut-off value with bootstrap support values shown at the nodes. The three includes representatives for the other genera included in Nitrospiraceae familiy (*Thermodesulfovibrio* and *Leptospirillum*) and is rooted at the *Thermodesulfovibrio* node.

Table 3
The factors that were tested to be correlated to microbial community composition in the biofilter biofilm and rearing water and water sump. The chemical parameters are measured in the water sump downstream the biofilter, except oxygen that was measured in the rearing tanks. *two different feed types (Ewos and Skretting).

Parameter	Biofilter biofilm (B—B)	Water rearing tank (W-T)	Water sump (W—S)
Fish presence	89%	100%	100%
Biomass (kg/m^3)	72%	83%	83%
Feed type*	77%	93%	77%
Oxygen saturation	28%	85%	88%
Mortality	62%	70%	65%
Salinity	17%	64%	30%
pH	19%	50%	2%
TAN	54%	63%	69%
NO_2^-	11%	9%	1%
NO_3^-	27%	19%	79%

and Verdegem, 2014) and oxidation of inorganic sulphur compounds (Molina-Muñoz et al., 2007). The significant different community compositions between water, biofilter biofilm and tank wall biofilm are in line with previous findings and expected due to different environmental selective pressures that are shaping the microbiota in RAS (Bakke et al., 2017; Rud et al., 2017; Bartelme et al., 2017; Duarte et al., 2019; Bartelme et al., 2019; Chen et al., 2019; Minich et al., 2020). Our results corroborate previous findings that the biofilter biofilm had higher Shannon's diversity than water (Rud et al., 2017; Bartelme et al., 2019; Aalto et al., 2022). In addition, tank wall biofilm had the had the lowest alpha diversity. Differences in community composition and alpha diversity can also be explained by different frequencies and methods of cleaning of the biofilm from biofilter and tank wall. The tank wall biofilm was thoroughly cleaned and had to go through a primary succession process between each production batch. The biofilter was backwashed regularly, without disinfection, which likely removed only the outer layer of the biofilm (Michaud et al., 2014) and had probably established a more diverse and mature biofilm in the deeper layers.

The community composition of both biofilm and water was surprisingly variable over time, compared to four commercial RAS producing salmon smolts monitored for the same period (Dahle et al., 2020b). The microbiota composition of biofilm and water differed significantly between fallowing and production periods. The impact of fish presence/absence is closely linked to feeding and organic matter load on the system and the carbon to nitrogen ratio (C/N ratio). Organic matter is typically the limiting resource determining the carrying capacity of the heterotrophic bacteria (Michaud et al., 2006) and is known to perturbate the microbial community structure and abundances in both biofilter and water (Michaud et al., 2006; Wold et al., 2014; Bartelme et al., 2017; Rojas-Tirado et al., 2018; Bartelme et al., 2019; Fossmark et al., 2020). During production batches the organic load increase, and consequently, the fraction of heterotrophic bacteria to nitrifying bacteria typically increase during production, which can impact

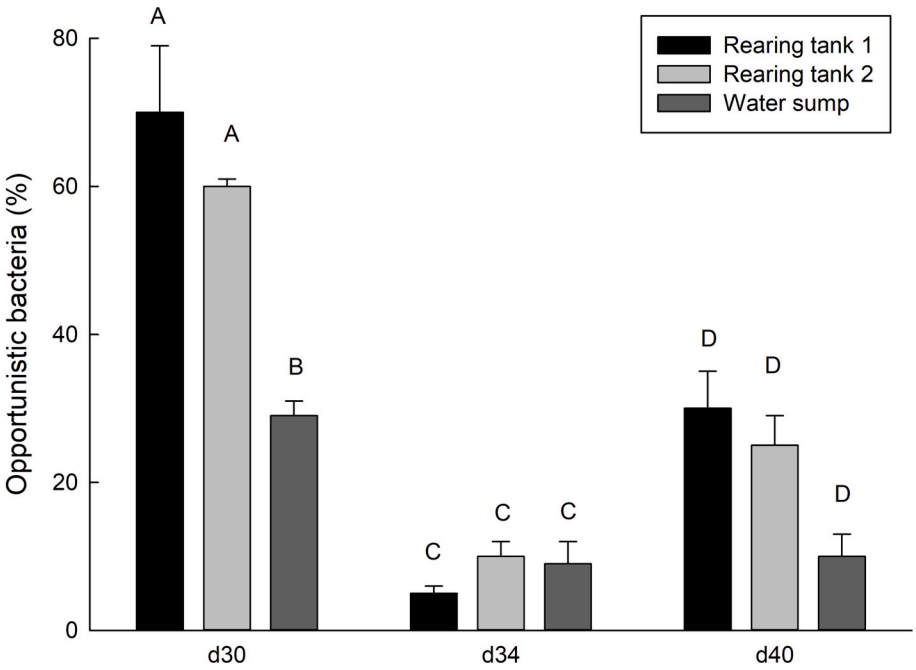

Fig. 12. The average fraction of rapid growing bacteria (±SE) in water samples from three different sampling sites; rearing tank 1, rearing tank 2 and water sump downstream the biofilter, in production batch 7, day 30, 34 and 40 in the production. The averages were calculated from three replicate water samples for each sample site on each sample day ± SE. Different letters indicate significant differences between the different samples at each sampling day.

Table 4
Average bacterial growth potential and total bacteria cells (±SE) at three different sampling sites and two different sampling dates in production batch 7, day 30 and 40. The average bacterial growth potential was calculated by dividing the increase in number of bacterial cells after three days incubation on agar by the total bacterial cells determined by flow cytometry. W-T = water rearing tank, W-S = water from sump, upstream disinfection. $n = 3$.

	Day 30			Day 40		
	W-T1	W-T2	W-S	W-T1	W-T2	W-S
Bacterial growth potential (%)	120 ± 11	131 ± 21	88 ± 7	706 ± 76	583 ± 33	488 ± 79
Total bacterial cells $\times 10^5$ mL^{-1}	5.0 ± 0.1	5.4 ± 0.1	5.1 ± 0.1	3.9 ± 0.2	4.0 ± 0.1	3.6 ± 0.2

nitrification negatively (Michaud et al., 2006; Michaud et al., 2014). Increased fraction of heterotrophic bacteria to nitrifiers was apparent in this study, as the relative abundance of OTUs representing nitrifiers decreased in abundance throughout the production batches and increased during fallowing periods. The fallowing periods were rather long (up to 40 days) and the dosing of ammonia was done to maintain the nitrifying bacteria active. We have observed highly stable community compositions in biofilter biofilm of RAS with shorter fallowing periods or continuous production during Atlantic salmon smolt production (Dahle et al., 2020b). We hypothesise that shorter fallowing periods or continuous production contributes to more stable conditions for the biofilter microbiota. A stable microbial community dominated by K-selected bacteria is suggested to indicate a more robust and resilient system against opportunistic and pathogen bacteria invasion and promote beneficial rearing conditions for the fish (Attramadal et al., 2012a, 2012b; De Schryver and Vadstein, 2014; Attramadal et al., 2014; Vadstein et al., 2018). On the contrary, alternation between production batches and fallowing can select for opportunistic bacteria that thrives under abrupt increases in organic loading (Attramadal et al., 2012b; Vadstein et al., 2018). However, importance of stable biofilter biofilm communities for optimal biofilter efficiency, microbial water quality and fish health is poorly understood and should be investigated closer in future research.

Supervised machine learning (SML) employing new learning algorithms has emerged as promising approach for data driven predictions and decision support in various disciplines (Pugliese et al., 2021). In this study we applied SML algorithms on amplicon sequencing derived OTU data and demonstrated that the composition of microbiota in both water and biofilter biofilm could predict presence of fish and fallowing periods in the system. The microbiota composition of water showed good predictability towards biomass of fish and oxygen saturation. In addition, the microbiota in the rearing tanks was a good predictor for feed type. The aforementioned variables are closely linked to each other and to fish presence and organic matter load in the system. The results shows that the presence of organic matter had a higher impact on the microbial communities than pH, salinity and nitrogen compounds in the studied RAS. However, the low correlation towards physicochemical parameters is most likely related to rather small variations during the monitored period, as it is well documented that for instance high fluctuations in salinity perturbates microbial communities in RAS (Bakke et al., 2017; Navada et al., 2019; Fossmark et al., 2021). So far there has been no published application of SML to microbial community data in RAS, but a good correlation between microbial communities and environmental impact around salmon net pens has been shown (Frühe et al., 2020). We have demonstrated here that SML models based on microbial communities could be used to predict fluctuations in RAS to a certain extent. SML has the potential to provide models that can predict instability or deteriorating conditions in RAS using microbial community dynamics.

An interesting observation was that although the microbial communities changed going from high to no load of organic matter during fallowing, it was changing back to a very similar composition during each production batch. This was especially evident for the water samples (Fig. 6B). The system seems to select in the same way for the suspended microbiota in each production batch and is likely a result of a similar selection pressure between production batches caused by system design and operational routines. The biofilter biofilm microbiota of the biofilter may also affect the microbial communities of the water (Dahle et al., 2022), but the knowledge on these interactions is limited (Rojas-Tirado et al., 2019). A selective exchange of bacteria is expected by released

bacteria from the biofilm to the water (Leonard et al., 2000; Michaud et al., 2009; Blancheton et al., 2013). Dahle et al. (2022) showed that the water microbiota developed differently in systems with immature biofilters compared to matured biofilters and suggested that the biofilm microbiota of the biofilter may affect the microbial communities of the water more heavily than season, fish size and management like disinfection. Our results along with others show that the microbial communities in the biofilter biofilm and rearing water were significantly different, but still share many abundant genera (Michaud et al., 2009; Bakke et al., 2017; Bartelme et al., 2019; Almeida et al., 2021) and generally follows similar trends of temporal dynamics (Fig. 9). The covariance in temporal dynamics and shared taxa indicate that the biofilter microbiota has a prominent role in shaping the suspended water bacterial communities in RAS. The biofilter may also act as a buffer to changes in the system where the heterotrophic populations have a high capacity to maintain the abundance of bacteria in the water in response to sudden increases of organic matter loading (Rojas-Tirado et al., 2019). The microbial composition of the water varied more over time than the biofilter biofilm, indicating that the bacterial populations in the water are more sensitive to variation in water quality and management than the more protected biofilm bacteria. This corroborates previous studies (Michaud et al., 2009; Bakke et al., 2017; Rud et al., 2017; Roalkvam et al., 2020).

Nitrifying bacteria constituted a small fraction of the biofilter community, with a maximum relative abundance of 12.5%, which is in line with other studies of RAS exhibiting good biofilter efficiency (Fossmark et al., 2021; Ribicic et al., unpublished results). The relative abundance of nitrifying bacteria varied both within and between production batches (Fig. 10). Nitrifying communities were dominated by *Nitrospira* which are commonly found in biofilters of fresh and brackish water RAS (Bartelme et al., 2017; Fossmark et al., 2021; Aalto et al., 2022; Ribicic et al., unpublished results), while the abundances of ammonium oxidising bacteria (AOB) were low. The low AOB:NOB ratio indicates the presence of comammox *Nitropspira* bacteria, capable of complete ammonia oxidising, belonging to the *Nitrospira* genus (Costa et al., 2006; van Kessel et al., 2015). The third most abundant *Nitrospira* OTU was related to *Candidatus Nitrospira nitrificans*, identified as a comammox *Nitrospira* in trickling filters in RAS (van Kessel et al., 2015). The low abundance of OTUs classified as AOBs could also be explained by the presence of ammonia oxidising archaea (AOAs), which has been identified in high abundances in RAS (Brown et al., 2013; Bartelme et al., 2017). The primers used in this study, were however not designed to target archaea. It is likely that the AOA are competing with comammox *Nitrospira* in RAS, especially at low ammonia substrate concentrations (Bartelme et al., 2019).

The studied RAS included full-flow UV disinfection of the water directly upstream of the rearing tanks. The fraction of fast growing, potentially opportunistic, CFUs were significantly higher in the rearing tanks than in the water sump upstream the disinfection on day 30 of batch 7 and considerably higher on day 40 (Fig. 12). In addition, the alpha diversity was significantly lower in the rearing tanks compared to the water sump on the same sampling days. Also, the rearing tanks had a higher bacterial growth potential than the water sump, which indicate that higher supplies of resources are available for bacterial growth following the disinfection (Hess-Erga et al., 2010), giving favourable conditions for opportunists. Significant regrowth and proliferation of opportunistic bacteria after disinfection has been reported for systems with long hydraulic retention time (HRT) in the rearing tanks (60 min and longer), such as in marine hatcheries. These communities are also characterized by low alpha diversities. Significant regrowth of bacteria following UV treatment have been shown to result in an altered microbial community composition with negative effects on marine larval health and survival (Attramadal et al., 2012b; Vadstein et al., 2018; Dahle et al., 2020; Teitge et al., 2020; Attramadal et al., 2021). However, the water microbiota composition and the total bacterial concentration was relatively similar between the rearing tanks and in the water sump in this study, as in a comparable study of a commercial RAS producing salmon fry (Dahle et al., 2022). The similarity between the two water locations can be explained by the short HRT in the rearing tanks (18–28 min), that prevented high regrowth of bacteria in the rearing tanks and therefore prevented large changes in composition through the system (Bakke et al., 2017; Dahle et al., 2022). In systems with short HRT in the rearing tanks, UV disinfection can be used to restrict bacterial density (Summerfelt et al., 2009) without compromising the microbial water quality in the rearing tanks (Dahle et al., 2022). However, in theory, a community with considerable potential for opportunistic regrowth might be vulnerable for pathogen invasion. It is likely that pathogens are present in RAS at low abundances at normal production (Michaud et al., 2009; Dahle et al., 2020b; Lewin et al., 2020) and that beneficial microbial communities suppress these pathogens from proliferation (Vadstein et al., 2018; Borges et al., 2021). No disinfection in the loop or disinfection before the biofilter instead of before the rearing tanks could lower the regrowth of opportunistic bacteria in the tanks, which can improve microbial water quality and provide a more resilient system against proliferation of pathogens. This is something that should be investigated in RAS with short HRT, like salmonid production, in the future.

5. Conclusions

Our study showed that the composition of both the water and biofilm microbiota in the commercial RAS varied over time, and that fallowing periods had a substantial effect on the microbial communities. However, the microbiota returned to similar compositions during all production periods, indicating a similar selection pressure shaped the system's microbiota during all production phases. Nitrifying communities were dominated by *Nitrospira*, and the third most abundant *Nitrospira* OTUs were related to the comammox *Nitrospira nitrificans*. Although the microbial communities in the biofilter biofilm and water were significantly different, they shared many common taxa and generally followed similar trends of temporal dynamics, which suggest an interaction between the biofilter biofilm and the suspended bacteria. CFU analysis showed that the fraction of rapid-growing bacteria was significantly higher in the rearing water than in the water sump upstream the UV disinfection, indicating that disinfection upstream the rearing tanks allowed for growth of opportunistic bacteria. The absence of an in-line disinfection step or placing the disinfection unit upstream the biofilter might provide better microbial water quality and a more resilient system against pathogen invasion.

Credit author statement

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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.

Data availability

Accession numbers are filled in

Acknowledgement

This research was funded by the Norwegian Seafood Research Fund, project "MonMic" (grant 901392), Research Council of Norway (grant 272400) and Norwegian salmon aquaculture industry. We would like thank Deni Koseto (SINTEF Industry) for sample logistics and the RAS facility for providing samples and production data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2022.739155.

References

Aalto, S.L., Syropoulou, E., de Jesus Gregersen, K.J., Tijrola, M., Pedersen, P.B., Pedersen, L.-F., 2022. Microbiome response to foam fractionation and ozonation in RAS. Aquaculture 550, 737846. https://doi.org/10.1016/j.aquaculture.2021.737846.

Almeida, D.B., Magalhaes, C., Sousa, Z., Borges, M.T., Silva, E., Blanquet, I., Mucha, A.P., 2021. Microbial community dynamics in a hatchery recirculating aquaculture system (RAS) of sole (*Solea senegalensis*). Aquaculture 539, 736592. https://doi.org/10.1016/j.aquaculture.2021.736592.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Aust. J. Ecol. 26, 32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x.

Attramadal, K.J.K., Salvesen, I., Xue, R., Øie, G., Størseth, T.R., Vadstein, O., Olsen, Y., 2012a. Recirculation as a possible microbial control strategy in the production of marine larvae. Aquac. Eng. 46, 27–39. https://doi.org/10.1016/j.aquaeng.2011.10.003.

Attramadal, K.J.K., Øie, G., Størseth, T.R., Alver, M., Vadstein, O., Olsen, Y., 2012b. The effects of moderate ozonation or high intensity UV-irradiation on the microbial environment in RAS for marine larvae. Aquaculture 330-333, 121–129. https://doi.org/10.1016/j.aquaculture.2011.11.042.

Attramadal, K.J.K., Truong, T.M.H., Bakke, I., Skjermo, J., Olsen, Y., Vadstein, O., 2014. RAS and microbial maturation as tools for K-selection of microbial communities improve survival in cod larvae. Aquaculture 432, 483–490. https://doi.org/10.1016/j.aquaculture.2014.05.052.

Attramadal, K.J.K., Minniti, G., Øie, G., Kjørsvik, E., Østensen, M., Bakke, I., Vadstein, O., 2016. Microbial maturation of intake water at different carrying capacities affects microbial control in rearing tanks for marine fish larvae. Aquaculture 457, 68–72. https://doi.org/10.1016/j.aquaculture.2016.02.015.

Attramadal, K.J.K., Øien, J.V., Kristiansen, E., Evjemo, J.O., Kjørsvik, E., Vadstein, O., Bakke, I., 2021. UV treatment in RAS influences the rearing water microbiota and reduces the survival of European lobster larvae (*Homarus gammarus*). Aquac. Eng. 94, 102176 https://doi.org/10.1016/j.aquaeng.2021.102176.

Badiola, M., Mendiola, D., Bostock, H., 2012. Recirculating aquaculture systems (RAS) analysis: Main issues on management and future challenges. Aquac. Eng. 51, 26–35. https://doi.org/10.1016/j.aquaeng.2012.07.004.

Bakke, I., Åm, A.L., Koleravic, J., Ytrestøyl, 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.

Bartelme, R.P., McLellan, S.L., Newton, R.J., 2017. Freshwater recirculating aquaculture system operations drive biofilter bacterial community shifts around a stable nitrifying consortium of ammonia-oxidizing archaea and Comammox *Nitrospira*. Front. Microbiol. 8, 101. https://doi.org/10.3389/fmicb.2017.00101.

Bartelme, R.P., Smith, M.C., Sepulveda-Villet, O.J., Newton, R.J., 2019. Component microenvironments and system biogeography structure microorganism distributions in recirculating aquaculture and aquaponic systems. mSphere 4, 1–15. https://doi.org/10.1128/mSphere.00143-19.

Bentzon-Tilia, M., Sonnenschein, E.C., Gram, L., 2016. Monitoring and managing microbes in aquaculture–towards a sustainable industry. Microb. Biotechnol. 9, 576–584. https://doi.org/10.1111/1751-7915.12392.

Blancheton, J.P., Attramadal, K.J.K., Michaud, L., Roque D'orbcastel, E., 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.

Borges, N., Keller-Costa, T., Sanches-Fernandes, G.M.M., Louvado, A., Gomes, N.C.M., Costa, R., 2021. Bacteriome structure, function, and probiotics in Fish Larviculture: the Good, the bad, and the gaps. Annu. Rev. Anim. Biosci. 9, 423–452. https://doi.org/10.1146/annurev-animal-062920-113114.

Boylen, E., Rideout, J.R., Dillon, M.R., Caporaso, J.G., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857. https://doi.org/10.1038/s41587-019-0209-9.

Bray, J.R., Curtis, J.T., 1957. An ordination of the upland Forest communities of Wisconsin. Ecol. Monogr. 27, 326–349. https://doi.org/10.2307/1942268.

Brown, M.N., Briones, A., Diana, J., Raskin, L., 2013. Ammonia-oxidizing archaea and nitrite-oxidizing nitrospiras in the biofilter of a shrimp recirculating aquaculture system. FEMS Microbiol. Ecol. 83, 17–25. https://doi.org/10.1111/j.1574-6941.2012.01448.x.

Chen, A., Chang, Z., Jiang, Y., Ge, H., Song, X., Chen, S., Zhao, F., Li, J., 2019. Effects of water recirculation rate on the microbial community and water quality in relation to the growth and survival of white shrimp (*Litopenaeus vannamei*). BMC Microbiol. 19, 192. https://doi.org/10.1186/s12866-019-1564-x.

Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. Austral. J. Ecol. 18, 117–143. https://doi.org/10.1111/j.1442-9993.1993.tb00438.x.

Cole, J.R., Wang, Q., Fish, J.A., Chai, B., McGarrell, D.M., Sun, Y., Brown, C.T., Porras-Alfaro, A., Kuske, C.R., Tiedje, J.M., 2014. Ribosomal database project: data and tools for high throughput rRNA analysis. Nucleic Acids Res. 42, 633–642. https://doi.org/10.1093/nar/gkt1244.

Cordier, T., Lanzen, A., Apotheloz-Perret, L., Stoeck, T., Pawlowski, J., 2019. Embracing environmental genomics and machine learning for routine biomonitoring. Trends Microbiol. 27, 397–398. https://doi.org/10.1016/j.tim.2018.10.012.

Costa, E., Pérez, J., Kreft, J.U., 2006. Why is metabolic labour divided in nitrification. Trends Microbiol. 14, 213–219. https://doi.org/10.1016/j.tim.2006.03.006.

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 biofilm. Aquaculture 552, 735097. https://doi.org/10.1016/j.aquaculture.2020.735097.

Dahle, S.W., Netzer, R., Lewin, A., Hageskal, G., Haugen, T., Ribicic, D., 2020b. Overvåking av bakteriesamfunn i settefiskproduksjon. In: En studie av fem RAS-anlegg (In Norwegian). SINTEF report 00470. https://www.fhf.no/prosjekter/prosjektbasen/901392/.

Dahle, S.W., Attramadal, K.J.K., Vadstein, O., Hestdahl, H.I., Bakke, I., 2022. Microbial community dynamics in a commercial RAS for production of Atlantic salmon fry (*Salmo salar*). Aquaculture 546, 737382. https://doi.org/10.1016/j.aquaculture.2021.737382.

Dalsgaard, J., Lund, I., Thorarinsdottir, R., Drenstig, A., Arvonen, K., Pedersen, P.B., 2013. Farming different species in RAS in Nordic countries: current status and future perspectives. Aquac. Eng. 53, 2–13. https://doi.org/10.1016/j.aquaeng.2012.11.008.

Davidson, J., Good, C., Williams, C., Summerfelt, S.T., 2017. Evaluating the chronic effects of nitrate on the health and performance of post-smolt Atlantic salmon *Salmo salar* in freshwater recirculation aquaculture systems. Aquac. Eng. 79, 1–8. https://doi.org/10.1016/j.aquaeng.2017.08.003.

De Schryver, P., Vadstein, O., 2014. Ecological theory as a foundation to control pathogenic invasion in aquaculture. ISME J. 8, 2360–2368. https://doi.org/10.1038/ismej.2014.84.

Duarte, L.N., Coelho, F.J.R.C., Oliveira, V., Cleary, D.F.R., Martins, P., Gomes, N.C.M., 2019. Characterization of bacterioplankton communities from a hatchery recirculating aquaculture system (RAS) for juvenile sole (*Solea senegalensis*) production. PLoS One 14, e0211209. https://doi.org/10.1371/journal.pone.0211209.

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996–998. https://doi.org/10.1038/nmeth.2604.

Edgar, R.C., 2016. SINTAX: a simple non-Bayesian taxonomy classifier for 16S and ITS sequences. BioRxiv 074161. https://doi.org/10.1101/074161.

Fossmark, R.O., Vadstein, O., Rosten, T.W., Bakke, I., Koseto, D., Bugten, A.V., Helberg, G.A., Nesje, J., Jørgensen, N.O.G., Raspati, G., Azrague, K., Østerhus, S.W., Attramadal, K.J.K., 2020. Effects of reduced organic matter loading through membrane filtration on the microbial community dynamics in recirculating aquaculture systems (RAS) with Atlantic salmon parr (*Salmo salar*). Aquaculture 524, 735268. https://doi.org/10.1016/j.aquaculture.2020.735268.

Fossmark, R.O., Attramadal, K.J.K., Nordøy, K., Østerhus, S.W., Vadstein, O., 2021. A comparison of two seawater adaption strategies for Atlantic salmon post-smolt (*Salmo salar*) grown in recirculating aquaculture systems (RAS): nitrification, water and gut microbiota, and performance of fish. Aquaculture 532, 735973. https://doi.org/10.1016/j.aquaculture.2020.735973.

Frühe, L., Cordier, T., Dully, V., Breiner, H.-W., Lentendu, G., Pawlowski, J., Martins, C., Wilding, T.A., Stoeck, T., 2020. Supervised machine learning is superior to indicator value inference in monitoring the environmental impacts of salmon aquaculture using eDNA metabarcodes. Mol. Ecol. 30, 2988–3006. https://doi.org/10.1111/mec.15434.

Guttman, L., van Rijn, J., 2008. Identification of conditions underlying production of geosmin and 2-methylisoborneol in a recirculating system. Aquaculture 279, 85–91. https://doi.org/10.1080/10454438.2020.1866733.

Hammer, Ø., Harper, D., Ryan, P., 2001. PAST-palaeontological statistics, ver. 1.89. Palaeontol. Electron. 4, 1–9.

Hess-Erga, O.-K., Blomvågnes-Bakke, B., Vadstein, O., 2010. Recolonization by heterotrophic bacteria after UV irradiation or ozonation of seawater; a simulation of ballast water treatment. Water Res. 44, 5439–5449. https://doi.org/10.1016/j.watres.2010.06.059.

Hjeltnes, B., Bæverfjord, G., Erikson, U., Mortensen, S., Rosten, T.W., Østergård, P., 2012. Risk Assessment of Recirculation Systems in Salmonid Hatcheries. VKM Report 2012: 01. ISBN: 978-82-8259-048-8.

Kolarevic, J., Baeverfjord, G., Takle, H., Ytterborg, E., Reiten, B.K.M., Nergård, S., Terjesen, B.F., 2014. Performance and welfare of Atlantic salmon smolt reared in recirculating or flow-through aquaculture systems. Aquaculture 432, 15–25. https://doi.org/10.1016/j.aquaculture.2014.03.033.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35 (6), 1547–1549. https://doi.org/10.1093/molbev/msy096.

Leonard, N., Blancheton, J.P., Guiraud, J.P., 2000. Populations of heterotrophic bacteria in an experimental recirculating aquaculture system. Aquac. Eng. 22, 109–120. https://doi.org/10.1016/S0144-8609(00)00035-2.

Letelier-Gordo, C.O., Aalto, S.-L.H., Suurnäkki, S., Pedersen, P.B., et al., 2020. Increased sulfate availability in saline water promotes hydrogen sulfide production in fish organic waste. Aquac. Eng. 89, 102062 https://doi.org/10.1016/j.aquaeng.2020.102062.

Lewin, A.S., Haugen, T., Netzer, R., Tondervik, A., Dahle, S.W., Hageskal, G., 2020. Multiplex droplet digital PCR assay for detection of *Flavobacterium psychrophilum* and *Yersinia ruckeri* in Norwegian aquaculture. J. Microbiol. Methods 177, 106044. https://doi.org/10.1016/j.mimet.2020.106044.

Llewellyn, M.S., Boutin, S., Hoseinifar, S.H., Derome, N., 2014. Teleost microbiomes: the state of the art in their characterization, manipulation and importance in aquaculture and fisheries. Front. Microbiol. 5 https://doi.org/10.3389/fmicb.2014.00207.

Martins, C.I.M., Eding, E.H., Verdegem, M.C.J., Heinsbroek, L.T.N., Schneider, O., Blancheton, J.P., d'Orbcastel, E.R., Verrath, J.A.J., 2010. New developments in recirculating aquaculture systems in Europe: a perspective on environmental sustainability. Aquac. Eng. 43, 83–93. https://doi.org/10.1016/j.aquaeng.2010.09.002.

Michaud, L., Blancheton, J.P., Bruni, V., Piedrahita, R., 2006. Effect of particulate organic carbon on heterotrophic bacterial populations and nitrification efficiency in biological filters. Aquac. Eng. 34, 224–233. https://doi.org/10.1016/j.aquaeng.2005.07.005.

Michaud, L., Giudice, A.L., Troussellier, M., Smedile, F., Bruni, V., Blancheton, J.P., 2009. Phylogenetic characterization of the heterotrophic bacterial communities inhabiting a marine recirculating aquaculture system. J. Appl. Microbiol. 107, 1935–1946. https://doi.org/10.1111/j.1365-2672.2009.04378.x.

Michaud, L., Giudice, A.L., Interdonato, F., Triplet, S., Ying, L., Blancheton, J.P., 2014. C/N ration-induced structural shift of bacterial communities inside lab-scale aquaculture biofilters. Aquac. Eng. 58, 77–97. https://doi.org/10.1016/j.aquaeng.2013.11.002.

Minich, J.J., Poore, G.D., Jantawongsri, K., Johnston, C., Bowie, K., Bowman, J., 2020. Microbial ecology of Atlantic Salmon (*Salmo salar)* hatcheries: impacts of the built environment on fish mucosal microbiota. Appl. Environ. Microbiol. 86, e0411–e0420. https://doi.org/10.1128/AEM.00411-20.

Molina-Muñoz, M., Poyotas, J.M., Vilchez, R., Hontoria, E., Rodelas, B., Gonzales-Lopez, J., 2007. Effect of the concentration of suspended solids on the enzymatic activities and biodiversity of a submerged membrane bioreactor for aerobic treatment of domestic wastewater. Appl. Microbiol. Biotechnol. 73 (6), 1441–1451. https://doi.org/10.1007/s00253-006-0594-0.

Navada, S., Vadstein, O., Tveten, A.-K., Verstege, G.C., Terjesen, B.F., Mota, V.C., Venkataraman, V., Gaumet, F., Mikkelsen, Ø., Kamstra, A., 2019. Influence of rate of salinity increase on nitrifying biofilms. J. Clean. Prod. 238, 117835 https://doi.org/10.1016/j.jclepro.2019.117835.

Navada, S., Vadstein, O., Gaumet, F., Tveten, A.-K., Spanu, C., Mikkelsen, Ø., Kolarevic, J., 2020a. Biofilms remember: osmotic stress priming as a microbial management strategy for improving salinity acclimation in nitrifying biofilms. Water Res. 176, 115732 https://doi.org/10.1016/j.watres.2020.115732.

Navada, S., Sebastianpillai, M., Kolarevic, J., Fossmark, R.O., Tveten, A.K., Gaumet, F., Mikkelsen, Ø., Vadstein, O., 2020b. A salty start: brackish water start-up as a microbial management strategy for nitrifying bioreactors with variable salinity. Sci. Total Environ. 739, 139934 https://doi.org/10.1016/j.scitotenv.2020.139934.

Nierychlo, M., Andersen, K.S., Xu, Y., Green, N., Albertsen, M., Dueholm, M.S., Nielsen, P.H., 2019. Species-level microbiome composition of activated sludge - introducing the MiDAS 3 ecosystem-specific reference database and taxonomy. Water Res. 182, 115955 https://doi.org/10.1016/j.watres.2020.115955.

Pugliese, R., Regondi, S., Marini, R., 2021. Machine learning-based approach: global trends, research directions, and regulatory standpoints. Data Sci. Manag. 4, 19–29. https://doi.org/10.1016/j.dsm.2021.12.002.

Roalkvam, I., Drønen, K., Dahle, H., Wergeland, H.I., 2020. A case study of biofilter activation and microbial nitrification in a marine recirculation aquaculture system for rearing Atlantic salmon (*Salmo salar* L.). Aquac. Res. 52, 94–104. https://doi.org/10.1111/are.14872.

Rojas-Tirado, P., Pedersen, P.B., Vadstein, O., Pedersen, L.-F., 2018. Changes in microbial water quality in RAS following altered feed loading. Aquac. Eng. 81, 80–88. https://doi.org/10.1016/j.aquaeng.2018.03.002.

Rojas-Tirado, P., Pedersen, P.B., Vadstein, O., Pedersen, L.-F., 2019. Microbial dynamics in RAS water: effects of adding acetate as a biodegradable carbon-source. Aquac. Eng. 84, 106–116. https://doi.org/10.1016/j.aquaeng.2018.12.010.

Ruan, Y.J., Guo, X.S., Ye, Z.Y., Liu, Y., Zhu, S.M., 2015. Bacterial community analysis of different sections of a biofilter in a full-scale marine recirculating aquaculture system. N. Am. J. Aquac. 77, 318–326. https://doi.org/10.1080/15222055.2015.1017128.

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.

Rurangwa, E., Verdegem, M.C.J., 2014. Microorganisms in recirculating aquaculture systems and their management. Rev. Aquac. 7 (2), 117–130. https://doi.org/10.1111/raq.12057.

Schreier, H., Mirzoyan, N., Saito, K., 2010. Microbial diversity of biological filters in recirculating aquaculture systems. Curr. Opin. Biotechnol. 21 (3), 318–325. https://doi.org/10.1016/j.copbio.2010.03.011.

Skjermo, J., Salvesen, I., Øie, G., Vadstein, O., 1997. Microbially matured water: a technique for selection of non-opportunistic bacterial flora in water that might improve performance of fish larvae. Aquac. Int. 5, 13–28. https://doi.org/10.1007/BF02764784.

Summerfelt, S.T., Sharrer, M.J., Tsukuda, S.M., Gearheart, M., 2009. Process requirements for achieving full-flow disinfection of recirculating water using ozonation and UV irradiation. Aquac. Eng. 40 (1), 17–27. https://doi.org/10.1016/j.aquae ng.2008.10.002.

Takahashi, S., Tomita, J., Nishioka, K., Hisada, T., Nishijima, M., 2014. Development of a prokaryotic universal primer for simultaneous analysis of Bacteria and Archaea using next-generation sequencing. PLoS One 9 (8), e105592. https://doi.org/10.1371/journal.pone.0105592.

Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10 (3), 512–526. https://doi.org/10.1093/oxfordjournals.molbev.a040023.

Teitge, F., Peppler, C., Steinhagen, D., Jung-Schroers, V., 2020. Water disinfection by ozonation has advantages over UV irradiation in a brackish water recirculation aquaculture system for Pacific white shrimp (*Litopenaeus vannamei*). J. Fish Dis. 43 (10), 1259–1285. https://doi.org/10.1111/jfd.13238.

Vadstein, O., Attramadal, K.J.K., Bakke, I., Olsen, Y., 2018. K-selection as microbial community management strategy: a method for improved viability of larvae in aquaculture. Front. Microbiol. 9, 2730. https://doi.org/10.3389/fmicb.2018.02730.

Vadstein, O., Bergh, Ø., Gatesoupe, F.-J., Galino-VIllegas, J., Mulero, V., Picchietti, S., 2013. Microbiology and immunology of fish larvae. Rev. Aquac. 5 (1), 1–25.

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, 555–559. https://doi.org/10.1038/nature16459.

van Rijn, J., Tal, Y., Schreier, H.J., 2006. Denitrification in recirculating systems: theory and applications. Aquac. Eng. 34, 364–376. https://doi.org/10.1016/j.aquaeng.2005.04.004.

Vestrum, R.I., Attramadal, K.J.K., Winge, P., Li, K., Olsen, Y., Bones, A.M., Vadstein, O., Bakke, I., 2018. Rearing water treatment induces microbial selection influencing the microbiota and pathogen associated transcripts of cod. Front. Microbiol. 9, 851. https://doi.org/10.3389/fmicb.2018.00851.

Wold, P.-A., Holan, A.B., Øie, G., Attramadal, K.J.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.

Zhu, S., Chen, S., 2001. Effects of organic carbon on nitrification rate in fixed film biofilters. Aquac. Eng. 25, 1–11. https://doi.org/10.1016/S0144-8609(01)00071-1.