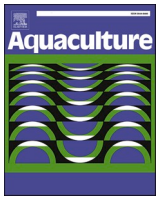
Aquaculture 546 (2022) 737382

Contents lists available at ScienceDirect

Aquaculture

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



Microbial community dynamics in a commercial RAS for production of Atlantic salmon fry (*Salmo salar*)

Stine Wiborg Dahle [a,*], Kari J.K. Attramadal [b], Olav Vadstein [b], Hans Ivar Hestdahl [c], Ingrid Bakke [b]

[a] SINTEF Ocean, Department of Aquaculture, 7465 Trondheim, Norway
[b] Department of Biotechnology and Food Science, NTNU Norwegian University of Science and Technology, 7491 Trondheim, Norway
[c] SalMar ASA, 7796 Follafoss, Norway

A B S T R A C T

Recirculating aquaculture systems (RAS) harbour complex microbial communities which can have an impact on the growth and development of the reared fish. This study aimed to improve our understanding of microbial community dynamics in a RAS involving three production batches of Atlantic salmon fry and parr during a period of 20 months. Water for analysis of microbiota was sampled at different positions in the RAS, and we also examined the effect of UV treatment on the water microbiota. Microbial communities were characterized by 16S rDNA amplicon sequencing of water samples taken directly upstream and downstream of the UV treatment unit and from three of the rearing tanks. In total 6 sampling events were made during a 20-month period. The study showed that: 1) Two of the production batches had a highly similar water microbiota despite disinfection of the system between the batches and rearing fish of different stages. In contrast, the first production batch showed a different water microbiota with variable composition through the system and over time. A more immature biofilter in the first batch may explain these differences. 2) The full-flow UV treatment directly upstream the rearing tanks had no observable effect on the community composition of the water microbiota in the different sampling positions in the RAS. This was likely a consequence of the low hydraulic retention time (HRT) (23 min) in rearing tanks, low bacterial regrowth in the fish tanks and community changes throughout the RAS loop. 3) The disinfection effect on viable bacterial densities in the water directly downstream of the UV treatment was around 89%, when the water was clear. Regrowth of bacteria following disinfection was low compared to those reported for marine RAS with UV disinfection and long HRT in fish tanks. The study shows that UV disinfection can be used to efficiently reduce bacterial density without compromising the microbial water quality in the fish tanks in RAS with low HRT.

1. Introduction

Recirculating aquaculture systems (RAS) have become a popular production system for Atlantic salmon (*Salmo salar*) (Badiola et al., 2012; Kolarevic et al., 2014; Rurangwa and Verdegem, 2015; Davidson et al., 2017). RAS provide several advantages compared to flow-through systems, like saving energy for heating, controlling and stabilizing water quality, and reducing environmental impact (Martins et al., 2010; Dalsgaard et al., 2013; Davidson et al., 2017). With a well-considered systems design, dimensioning and management strategies, RAS also have properties that can contribute to stable and mutualistic fish-microbe interactions (Skjermo et al., 1997; Attramadal et al., 2012a, 2012b, 2014; Attramadal et al., 2016; Vadstein et al., 2018; Vestrum et al., 2018; Duarte et al., 2019).

The microbial communities in RAS are complex and essential for both chemical and microbial water quality and plays a crucial role for the health of the cultured fish (Blancheton et al., 2013; Vadstein et al., 2018). Certain microbial assemblages may impact fish health positively, others may have a negative influence on the fish, and even cause mortality. The microbial communities in RAS are affected by feed and feeding regimes, the make-up water, management routines, the fish itself and selection pressure in the system (Attramadal et al., 2012a; Blancheton et al., 2013; Vadstein et al., 2018). Hence, the microbial assemblages can vary between systems and over time (Rud et al., 2017; Bakke et al., 2017; Dahle et al., 2020; Fossmark et al., 2020a, 2020b). Unstable microbial water quality with high fractions of opportunistic bacteria, is one important factor that contributes to sub-optimal conditions for the cultured fish (Bakke et al., 2017). However, the mechanisms causing these changes are fairly well understood for marine larvae (Vadstein et al., 2018), but poorly documented for salmonids. Thus, more knowledge is needed to understand and improve microbial management strategies specifically for land-based cultivation of salmonids.

Disinfection of the intake water reduces the risk of entry and spreading of pathogens into the system (Sharrer et al., 2005; Summerfelt

* Corresponding author at: SINTEF Ocean, Department of Aquaculture, Brattørkaia 17C, 7465 Trondheim, Norway.
E-mail address: Stine.w.dahle@sintef.no (S.W. Dahle).

https://doi.org/10.1016/j.aquaculture.2021.737382
Received 13 May 2021; Received in revised form 20 August 2021; Accepted 21 August 2021
Available online 24 August 2021

et al., 2009), and is of paramount importance for the biosecurity of land-based facilities. However, opportunistic and pathogenic bacteria may still reside among the bacteria inside the RAS, and disinfection can be used to continuously disinfect recirculated water before it returns to the rearing tanks (Summerfelt et al., 2009). Experiments with in-line UV disinfection in pilot scale RAS have shown a reduction of heterotrophic bacteria by 98% (Huyben et al., 2018) and a lower microbial activity (Huyben et al., 2018; Schumann and Brinker, 2020; de Jesus Gregersen, 2020) . UV disinfection also reduces the micro particle numbers by destroying bacteria that uses micro particles as substrate and surface area (Pedersen et al., 2017; Gregersen et al., 2020).

Disinfection kills and inactivates bacteria but does not reduce the amount of substrate available for bacterial growth. Disinfection therefore leads to a situation of low competition for the available substrate, and therefore favour r-selection and subsequent proliferation of opportunistic bacteria in the rearing water (Sharrer et al., 2005; Hess-Erga et al., 2010; Attramadal et al., 2012b; Vadstein et al., 2018; Attramadal et al., 2021). The time window between disinfection and significant bacterial regrowth is determined by the number and growth rates of bacteria surviving or seeding the water volume from biofilm following disinfection. UV disinfection within the RAS treatment loop, and especially immediately before the fish tanks, is therefore hypothesized to constitute a disadvantage for the health of fish in an otherwise well dimensioned and managed RAS (Attramadal et al., 2012b; Vadstein et al., 2018; Dahle et al., 2020; Attramadal et al., 2021). Several experiments with marine larvae have shown that UV treatment inside the RAS loop destabilise the microbial composition of the rearing with negative effects on viability and survival (Attramadal et al., 2012b; Dahle et al., 2020; Attramadal et al., 2021). However, the effects of UV irradiation in commercial freshwater RAS for salmon production on microbial community composition and blooms of opportunistic bacteria is not studied.

Here, we characterize the water microbiota in a commercial RAS for production of Atlantic salmon fry and parr by sequencing of 16S rRNA amplicons. We sampled five positions in the RAS loop and covered three distinct production batches over a 20 months' period. The aim was to map the temporal dynamics of the water microbiota in this commercial system over a long-term period, to elucidate the impact of UV disinfection on the water microbiota, and to improve the general understanding of the bacterial community dynamics in RAS.

2. Methods

2.1. Culture system

The study was based on sampling of water in a commercial RAS for production of Atlantic salmon fry and parr in Norway. The start-feeding RAS department (Inter Aqua Advance, Denmark) consisted of 18 octagonal fish tanks (16 m^3) operated with fresh water (Fig. 1). The intake water was from a hydroelectric power plant and was prefiltered with a coarse screen, without UV treatment. The volume of the total system was 470 m^3 and the system flow set to 10.8 m^3/min. The RAS included the following components after the fish tanks: a drum filter of 60 μm, pH-regulation, two Moving Bed Biofilters (MBBF) (Inter Aqua Advance,with Curler advance X-1 bioelement, volume: 2 × 50 m^3), two drum filters (60 μm), two Fixed Bed Biofilters (FBBF) (Inter Aqua Advance), Trickling filter (Inter Aqua Advance) and a UV unit (Atlantium RZ 2300–12, Teknor, Norway) with a dose of 75–100 mJ/cm^2, treating the full-flow right before entering the fish tanks (Fig. 1). The light regime was 24:0 with fluorescent tubes. Hydraulic retention time was 23 min in the fish tanks and in average 7–9 days for the total system. The make-up water flow varied during the production and between batches of fish, from 0.15–3.8 L $h^{-}1$ for the spring batches and 0.7–4.81 L h^{-1} for the autumn batch, amounting to 300–400 L new water/kg feed. The system, included the biofilter, was disinfected before 2015_spring and between batch 2015_autumn and 2016_spring, in consecutive treatments with lye, chlorine dioxide and ozone. Before the 2015_spring batch the biofilter was started after disinfection with new, clean carriers and maturated until the stocking of fish, while before the 2016_spring batch the biofilter included already matured biofilm carriers from an already running biofilter. The 2015_autumn batch was not disinfected before stocking of fish. Production data and physicochemical water quality variables were provided by the RAS facility for the periods, including temperature, total ammonia nitrogen (TAN), nitrite, nitrate, CO_2, alkalinity, and pH. Normally, four to five batches of fish are produced during a year in this system.

2.2. Rearing regime

In this study, we monitored three different batches of fish in the same system. The spring production batches growing fish up to 3–4 g in 2015 (2015_spring) and 2016 (2016_spring) took place during the months of February, March, and April. The autumn 2015 production batch growing fish up to 25 g took place during September, October, and

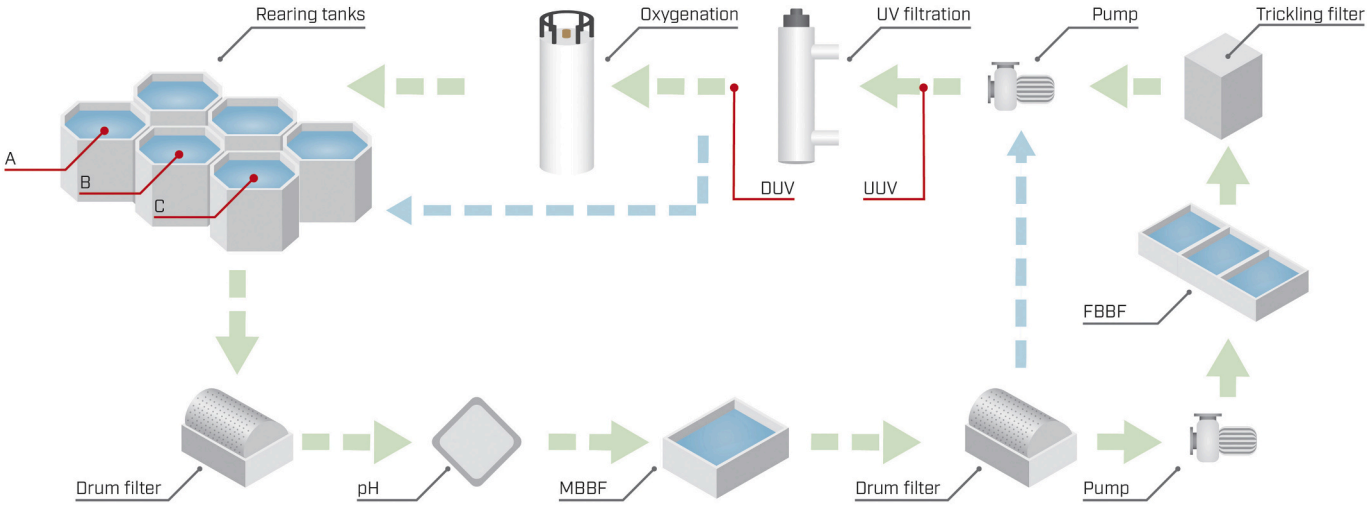


Fig. 1. Schematic presentation of the RAS unit monitored in the study. Samples for analyses of the RAS water microbiota (red lines) were taken from three fish tanks (A, B, C), and immediately upstream (UUV) and downstream of the UV treatment unit (DUV). MBBF = Moving Bed Biofilter, FBBF=Fixed Bed Biofilter. The UV disinfection represent a full-flow disinfection. Illustration by Mats Mulelid, SINTEF Ocean.

November (Fig. 2). The final biomass density in each tank was 37–48 kg/m^3 for the spring batches and 65–71 kg/m^3 for the autumn batch. Dead fish were removed and recorded daily to assess the daily mortality in each tank. The spring batches were fed continuously Ewos Micro 040 and 1 (Ewos, Norway) while the autumn batch was fed Ewos Micro 5 and 15, according to fish size. Feed load pr day during the period was 0.7–19 kg/tank for the spring batches and 5.6 to 25.0 kg/tank for the autumn batch.

2.3. Sampling for microbiological analysis

For characterization of the bacterial communities by DNA-based 16S rDNA amplicon sequencing, water directly upstream (UUV) and downstream of the UV unit (DUV) and from three rearing tanks (A, B, C) was sampled one to three times during the three production batches (Fig. 2). The 2015_spring batch was first sampled two days prior to the inset of fish (d-2) and at day 12 and 34. The 2015_autumn batch was sampled once, at day 29, and the 2016_spring batch was sampled at day 13 and 41 after inset of fish. Water samples were also collected for RNA-based 16S rRNA amplicon sequencing for 2015_autumn on day 29 and 2016_spring on day 41. The water samples were filtrated using Dynagard filters (pore size 0.2 μm, Microgon) and Omnifix® syringes. Around 150–200 mL water was filtrated for each water sample. Samples were frozen (−20 °C) immediately after sampling, transported to NTNUs laboratory and stored at −80 °C until further processing.

Water samples for quantification of colony forming units (CFU) were collected from the same positions in the RAS at selected sampling times during the production batches 2015_spring (day −2 and 34) and 2016_spring (day 13 and 41). Approximately 1 L of water was collected from each sampling position in triplicates and mixed well before 1 mL was used in CFU analysis as described below.

2.4. Microbial community analyses

To quantify CFU in water samples serial dilutions of the sampled water (1:10–1:1000) were prepared and streaked out on M65 agar plates with 0.1% NaCl (0.5 g/L peptone, 0.5 g/L tryptone, 0.5 g/L yeast extract, and 1 g NaCl per L water) in triplicates for each dilution. The CFU numbers were determined as the number of colonies observed after 14 days' incubation in room temperature.

For characterization of bacterial community composition, DNA was extracted using the Power Soil DNA isolation (MOBIO) as described by the manufacturer. For two sampling times, the PowerMicrobiome RNA Isolation Kit (MOBIO) was used to extract total RNA water samples, as following the protocol. cDNA was synthesized by use of Prime ScriptTM 1st strand cDNA Synthesis Kit (TaKaRa), as described by the manufacturers. Random 6 mers and approximately 1 mg total RNA was used as template in each reaction. The third and fourth variable regions (V3, V4) of the bacterial 16S rRNA gene was amplified from DNA extracts and cDNA using the primers Ill-338F (5′- TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNCCTACGGGWGGCAGCAG) and Ill-805R (5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNNGACTACNVGGGTATCTAAKCC) (Nordgård et al., 2017) and Phusion Hot Start DNA Polymerase (Thermo Scientific, USA). The amplicons were normalized using the SequalPrepTM Normalization Plate Kit (Invitrogen, USA) and indexed using the Nextera XT Index kit (Illumina, USA) as described in Vestrum et al. (2020). Amplicons were pooled and concentrated using the Amicon Ultra-0.5 Centrifugal Filter Device, and the resulting amplicon library was sequenced on an MiSeq run (Illumina, USA) with the MiSeq Reagent Kit v3 in the 2 × 300 bp paired-end mode at the Norwegian Sequencing Centre. The resulting sequencing data are deposited at the European Nucleotide Archive (accession numbers ERS7273454 - ERS7273493).

The Illumina sequencing data were processed using the USEARCH pipeline (version 11; https://www.drive5.com/usearch/). The command Fastq_mergepairs was used for merging of paired reads, trimming off primer sequences and filtering out reads shorter than 400 base pairs. Further processing included demultiplexing and quality trimming (the Fastq_filter command with an expected error threshold of 1). The UPARSE-OTU algorithm (Edgar, 2013) was used for chimera removal and clustering at the 97% similarity level. Taxonomy assignment was performed applying the Sintax script (Edgar, 2016) with a confidence value threshold of 0.8 and the RDP training set (version 16). The resulting OTU (operational taxonomic units) table was normalized to 37,000 number of reads (the lowest number of reads obtained among samples) per sample by determining the fraction of the OTUs for each community profile, and then multiplying with 37,000, and finally rounding off the read numbers to integers. 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.

2.5. Statistical analysis

The data for fish survival were Arcsin-transformed before statistical analysis by one-way ANOVA (SPSS version 27). For comparisons of chemical variables and microbiota, One-way ANOVA or Kruskal-Wallis were used, depending on normality and homogeneity of variance of the variables (SPSS). Statistical analyses of microbial community data, based on the OTU table, were performed using the program package PAST (version 3; Hammer et al., 2001). OTUs with a maximum abundance of less than four reads in all samples in the normalized OTU table (37,000 reads per sample) were filtered out of the OTU table prior to multivariate analyses. Principal coordinate analysis (PCoA) (Davis, 1986) was based on Bray-Curtis similarities (Bray, 1957). To test for differences in community structure between sample groups, we applied one-way PERMANOVA based on Bray-Curtis similarities (Anderson, 2001). The Similarity Percentages (SIMPER) analysis (Clarke, 1993) was used to determine the contribution from the OTUs to the Bray-Curtis dissimilarity between samples and groups.

3. Results

Three different batches of fish were produced in different periods in the same RAS unit during this study. The batch 2015_spring and 2016_spring both produced fry (from 0.2 to 4 g) in the same period of the year (February, March, April) in two subsequent years, whereas the 2015_autumn batch produced parr (from 2.5 to 25 g) during September,

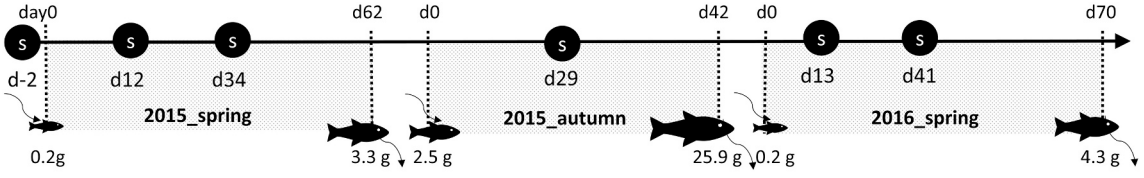


Fig. 2. Timeline for sampling of microbiota and production at the RAS facility with weight of fish in grams. 2015_spring, 2015_autumn and 2016_spring indicates the three production batches that were monitored. Production batches in spring were produced from February to April and production batch autumn from September to November. S = sampling, d = day of production (day 0 corresponds to the day of inset of fish in the RAS), sampling d-2 = two days before fish were put in the RAS unit.

October, and November.

3.1. Physicochemical water quality

The average physiochemical water quality variables were generally satisfying according to the recommended thresholds for Atlantic salmon and relatively similar among the three production batches examined (Table 1). However, the 2015_autumn production batch had higher NO_3^--N concentrations than the other batches, and 2016_spring had higher total ammonia nitrogen concentration. None of these differences were statistically significant.

3.2. Fish performance

The average mortality of fish in batch 2015_autumn (0.01 ± 0.00%) was significantly lower compared to that in batch 2015_spring (0.05 ± 0.01%) and 2016_spring (0.06 ± 0.01%) (Kruskal-Wallis, $p = 0.001$; 0.001). The mortality was also more stable over time for batch 2015_autumn, compared to the batches from spring, which had peaks in mortality during the production (Fig. 3). 2015_spring had a maximum mortality of 0.60% at day 23, whereas for 2016_spring the highest mortality was 3.12% at day 16. The following days the mortality decreased and was relatively stable throughout the batches.

3.3. Microbial water quality

3.3.1. Microbial community composition in the RAS water

The relative abundance of the bacterial orders differed among the production batches. The water from batch 2015_spring had a clearly different community composition compared to the other batches (Fig. 4). The most abundant bacterial order in rearing water for 2015_spring was Pseudomonadales, which was also significantly more abundant in 2015_spring than in the other two batches (Kruskal-Wallis, $p = 0.001$; 0.001). For 2015_spring samples Pseudomonadales accounted for 20–81% of the community, with an average of 46%. For the other production batches this order constituted 1–28%, with an average of 8%. In contrast, Burkholderiales was the most abundant order for production batch 2015_autumn and 2016_spring, with abundance ranging from 19 to 38%, and with an average of 28%. Rhodobacterales and Cytophagales were considerably more abundant in the water during the 2015_autumn and 2016_spring batches compared to the 2015_spring batch (Fig. 4). Ordination by Principal Coordinate Analysis (PCoA) indicated that that the bacterial community composition clustered according to batch and sampling time (Fig. 5). A PERMANOVA test confirmed that the water microbiota was significantly different between production batches ($p = 0.02$). Even though batch 2016_spring was produced in the same season as 2015_spring, with the same size of fish and operated similarly, the composition of the microbiota was more similar to production batch 2015_autumn. The autumn batch was produced during another season, with bigger size of fish (Fig. 5). Average Bray-Curtis similarities showed that the community composition for production batch 2015_spring was considerably more variable between fish tanks and over time compared to batch 2015_autumn and 2016_spring (Fig. 6).

Table 1
Physicochemical water quality measured in the RAS loop (after the water treatment) during the production batch (mean ± SE)).

	2015_spring	2015_autumn	2016_spring
Temperature (°C)	13.70 ± 0.05	13.20 ± 0.09	14.10 ± 0.05
Total ammonia nitrogen (mg TAN L^{-1})	0.90 ± 0.52	0.73 ± 0.28	1.39 ± 0.33
Nitrite (mg NO_2-N L^{-1})	0.31 ± 0.16	0.23 ± 0.09	0.26 ± 0.24
Nitrate (mg NO_3-N L^{-1})	15.16 ± 3.74	25.54 ± 2.10	9.00 ± 7.07
CO_2 (mg L^{-1})	13.28 ± 1.51	15.36 ± 1.45	14.47 ± 1.44
Alkalinity (mg $CaCO_3$ L^{-1})	1.19 ± 0.02	1.41 ± 0.01	1.33 ± 0.01
pH	7.17 ± 0.02	6.97 ± 0.03	7.10 ± 0.01

Number of measurements done during production equal 42 to 70.

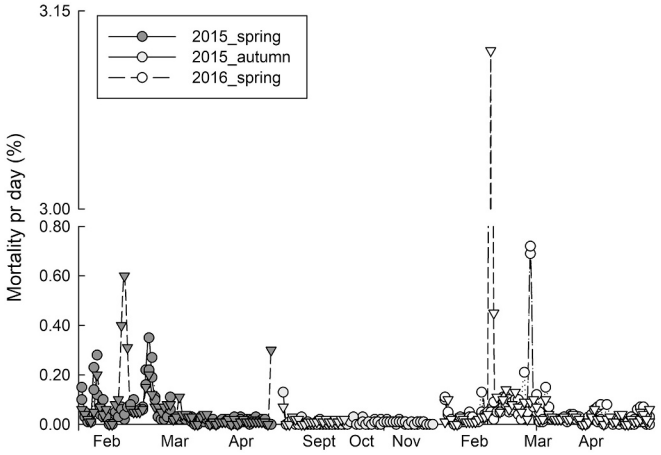


Fig. 3. Fish mortality during the three different batches of fish production (2015_spring, 2015_autumn and 2016_spring) from three different fish tanks (square, circle and triangle represent the three different fish tanks).

The most abundant OTU of the whole dataset, OTU_8 (*Pseudomonas*) was significantly more abundant in the 2015_spring batch than in the two other batches (Kruskal-Wallis, $p < 0.05$), with average abundances of 16% of the total reads for the 2015_spring samples, compared to only 0.005 and 0.07% for the 2015_autumn and the 2016_spring batch, respectively (Table 1, Supplementary). The genus *Acinetobacter* (Pseudomonadales) was represented by three OTUs (OTU 1, 2 and 14) that were significantly more abundant in the 2015_spring samples (Kruskal-Wallis, $p < 0.05$), with average abundances of 7% (Fig. 1, Supplementary). In the 2016_spring samples, OTU_3 (*Rhodobacteraceae*), was significantly more abundant (average abundance 10.4%) than in the 2015_spring and 2015_autumn samples (Kruskal-Wallis, $p < 0.05$). For the 2015_autumn samples, the most abundant OTU was OTU_10 (*Enterobacteriaceae*; average abundance 8%) which was considerably higher compared to the other batches (Table 1, Supplementary).

The alpha diversity of the RAS water microbiota, expressed as OTU Richness and the exponential Shannon's diversity index ($e^{Shannon's}$), was considerably higher for production batch 2015_autumn (only one sampling time) than for the water of the spring batches (Fig. 7A, B), and were significantly higher than 2015_spring (Kruskal-Wallis, p = 0.001). No significant differences in alpha diversity were detected between the 2015_autumn and 2016_spring batches. Generally, the diversity increased with time for the spring batches (Fig. 7A, B). The alpha diversity of the water microbiota was slightly reduced in the fish tank compared to the rest of the system (Fig. 7), which may be a consequence of regrowth. The differences between upstream/downstream the UV and the fish tanks were however not significant.

3.3.2. Temporal dynamics of the water microbiota

The community composition for the water of production batch 2015_spring was substantially more variable over time, compared to the 2016-spring batch (Fig. 6). Both the PCoA ordination (Fig. 5) and the average Bray-Curtis similarities (Fig. 6) demonstrated that particularly in the 2015_spring batch, the water microbiota underwent major changes throughout the production period (Fig. 6). The relative abundance of OTUs representing *Acinetobacter* (OTU 1, 2 and 14) increased with sampling time (Supplementary, Table S1, Fig. S1), and contributed with 23.6% of the differences between timepoints (SIMPER analysis). The Bray-Curtis similarities for comparison of the tank water microbiota between sampling times were high for 2016_spring (Fig. 6) and indicated stability of the water microbiota throughout this production batch.

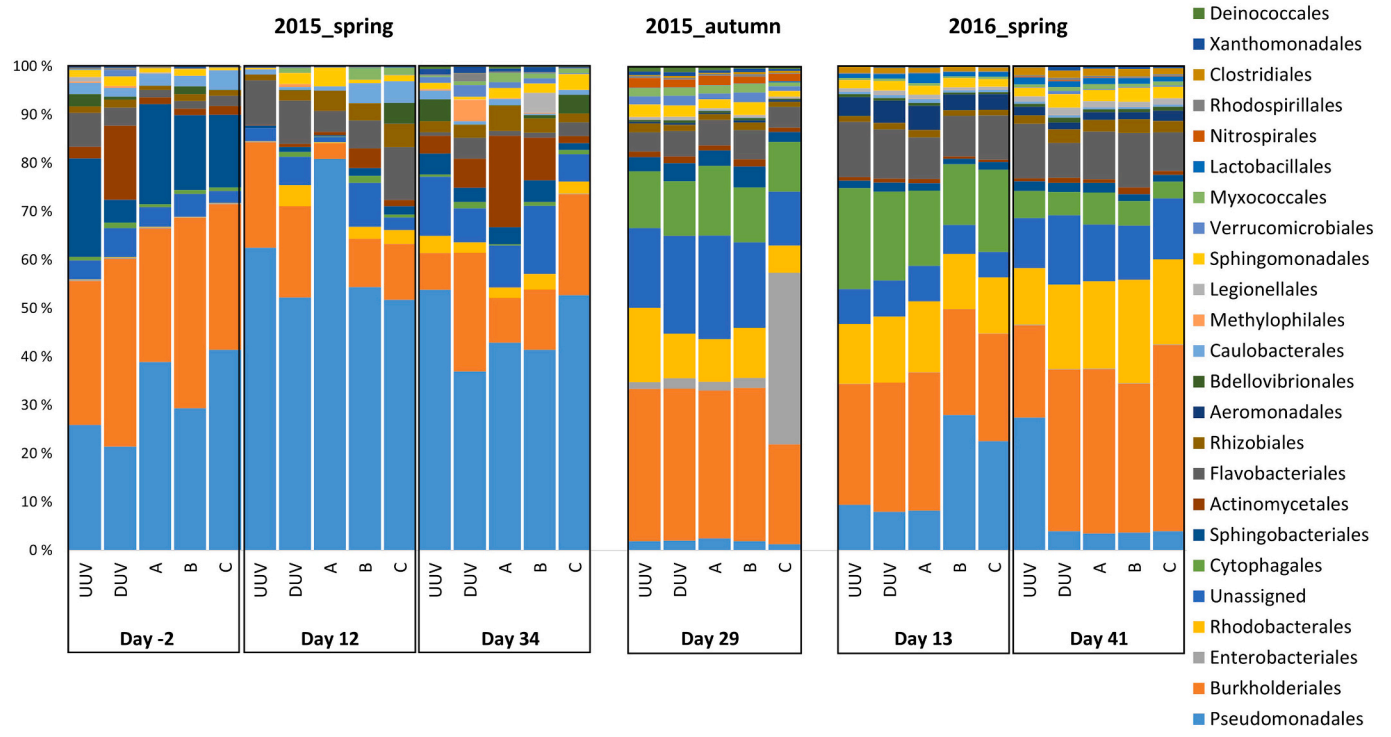


Fig. 4. Relative abundances (%) of bacterial orders in the rearing water of the different batches of fish (2015_spring, 2015_autumn, 2016_spring), at different sampling days, where day represent the day in production and − 2 represent two days before inset of fish. UUV = upstream UV, DUV = downstream UV. A, B and C = three different rearing tanks. Only orders that are present at abundances >1% in at least one sample are shown.

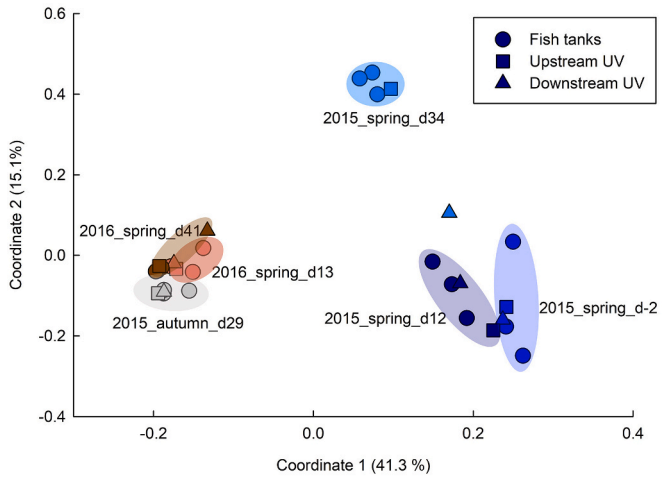


Fig. 5. PCoA plot based on Bray–Curtis similarities for three different batches of fish (2015_spring, 2015_autumn, 2016_spring), at different sampling days (d), where d-2 represent two days before inset of fish. Samples include tank water microbiota and water upstream and downstream the UV treatment.

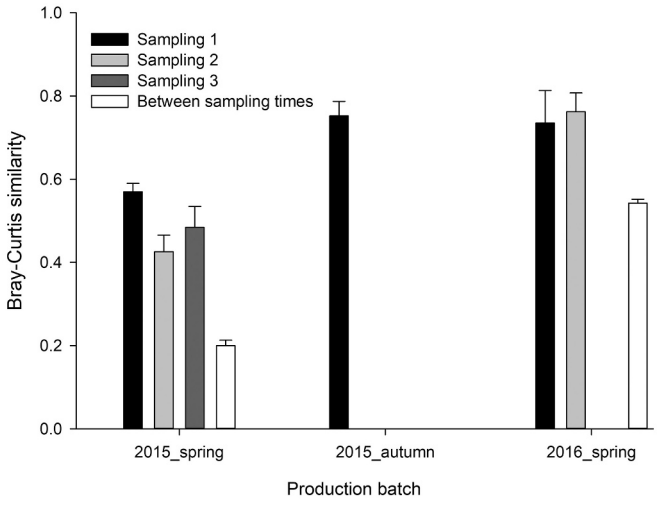


Fig. 6. Bray–Curtis similarities for comparing the water microbiota within each production batch at sampling 1, 2 and 3 and between sampling times. At each sampling time, the samples include upstream and downstream of the UV treatment and the three rearing tanks. Sampling days for each production batch are presented in Fig. 2. Error bars show the standard error (±SE) of all the samples, $n = 5$.

3.3.3. *Microbial community dynamics throughout the RAS*

The PCoA plot (Fig. 5) indicated that in general, the water microbiota was relatively similar between sampling points throughout the system on the same sampling day. This was particularly evident for Bray-Curtis similarities for the two last batches (Fig. 6). DNA-based methods include live, inactivated, and dead bacterial cells. To improve the possibility to detect changes in the active microbial communities, and to reveal potential effects of UV treatment on the water microbiota, we performed 16S rRNA amplicon sequencing based on total RNA extracts for water samples from 2015_autumn (day 29) and 2016_spring (day 41). However, neither PCoA ordinations with Bray-Curtis (Fig. 8) nor Dice-Sørensen coefficients (data not shown) indicated larger variation in community composition throughout the system for RNA-based compared to DNA-based analyses. Still, there was significant differences between the DNA and RNA based analyses (PERMANOVA; $p = 0.01$; $p = 0.008$).

We were particularly interested in the effect of the UV treatment on the water microbiota, but there were no indications this had any observable effect, judged on comparison of the DNA- and RNA-based community composition using ordination. However, a manual

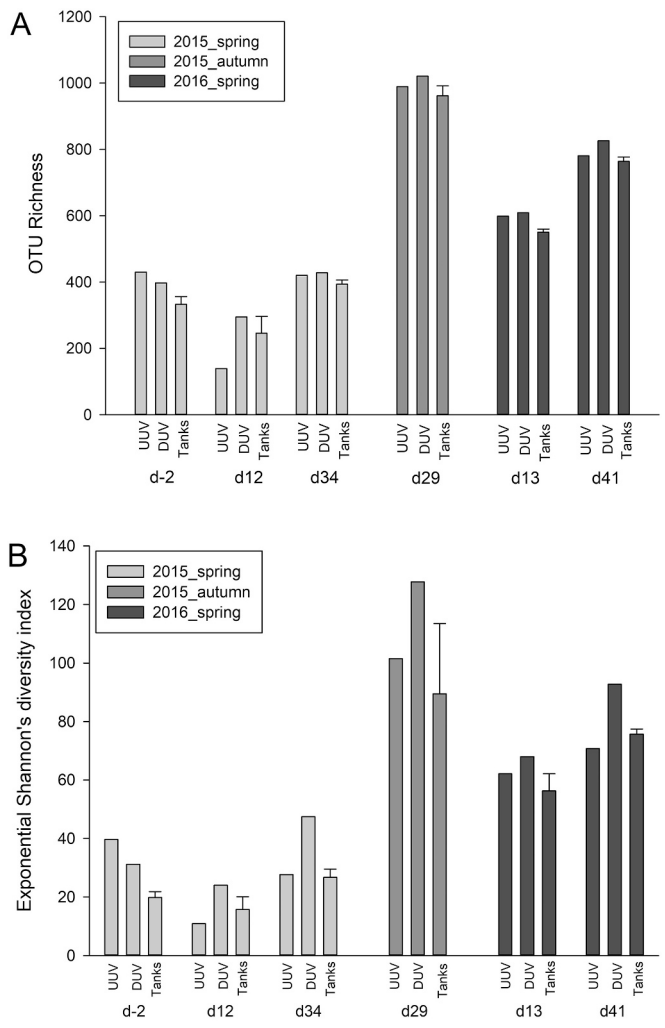


Fig. 7. Alpha diversity of RAS water expressed as A) OTU Richness and B) Exponential Shannon's diversity index, at different sampling days (d). Error bars show the standard error (±SE) between triplicate fish tanks. UUV = upstream UV, DUV = downstream UV. d-2 represent two days before inset of fish.

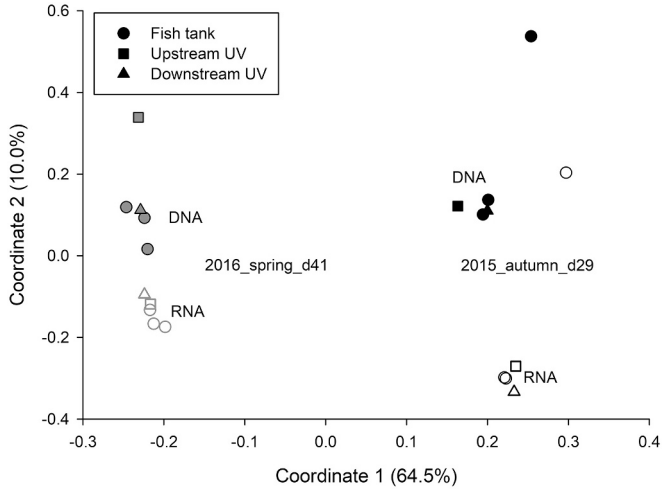


Fig. 8. PCoA plot based on Bray–Curtis similarities for DNA- (closed symbols) and RNA-based (open symbols) samples. Samples include microbiota from tank water and water upstream and downstream the UV treatment at two different sampling dates (2016_spring day 41 and 2015_autumn, day 29). d represent the day in production.

inspection of the OTU table revealed that three OTUs (OTU 1, 2 and 14) had a lower abundance in samples taken downstream of the UV treatment compared to upstream the UV treatment. The RNA-based data showed that these OTUs increased in the fish tanks (Supplementary Table 1, Fig. 1). These OTUs were all classified as *Acinetobacter* and were the same OTUs that were highly abundant in the 2015_spring samples (Supplementary Table 1, Fig. 1).

We also examined the effect of the full-flow UV treatment on the culturable, living bacterial cells as CFU for water samples from the two spring batches (Fig. 9). The number of CFU was lowest for the water samples taken downstream of the UV treatment for all samplings. Samples from batch 2015_spring (d-2, and d34), and the d13 sample from batch 2016_spring had a reduction of 86.7 to 91.0% in CFU after the UV treatment. In comparison, day 41 of batch 2016_spring showed a more turbid water with a higher concentration of bacteria upstream the UV (Fig. 9) and a 20.1% reduction of CFU by the UV. For all samplings, the CFU increased when the UV treated water entered the rearing tanks, indicating regrowth of bacteria (Fig. 9). The increase in CFU from the fish tanks to the UV treatment, indicates bacterial regrowth throughout the entire system. With exception of the 2016_spring_d41 samples, the CFU counts increased with a magnitude of 4 times in the fish tanks, and 3 times in the water treatment loop. This estimate includes samples from batch 2015_spring taken prior to the introduction of fish, when the regrowth was lower compared to the other sample dates batches. For day 41 of 2016_spring, however, when the UV treatment was suboptimal due to a high visually observed turbidity, the regrowth of bacteria was higher in the fish tanks than through the subsequent water treatment loop.

4. Discussion

Despite progress, there is still limited information available on microbial community dynamics in RAS producing salmonids, although these communities can have a large impact on the health of the fish (Blancheton et al., 2013; Vadstein et al., 2018). Increased knowledge about microbial communities in RAS is important for improved operational design, management, and a sustainable production. One approach to this knowledge is to study commercial RAS. These studies often lack an experimental control and replicates, which can be challenging in commercial systems. Also, the physicochemical parameters vary a lot during production. However, the effects of water treatment processes can be studied by sampling upstream and downstream a treatment unit. If such studies are repeated in time, the conclusions are more robust than

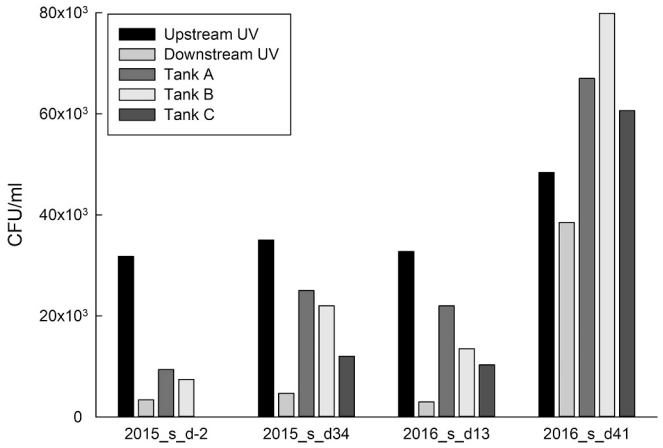


Fig. 9. Bacterial densities determined as CFU/ml in water upstream (UUV) and downstream the UV treatment (DUV) and in the fish tanks (Tank A, B and C) for two sampling dates for batch 2015_spring (d-2 and d34) and 2016_spring (d13 and d41). d represent the day in production, where −2 is two days before inset of fish to the RAS unit.

experiments with replication at the same sampling. Such studies also complement controlled experimental systems by extracting information at the relevant scale.

This study aimed to improve the understanding of microbial community dynamics in a commercial RAS during a period of 20 months and included three production batches of Atlantic salmon fry and parr. Water was sampled at different positions through the system, to examine the community dynamics and the effect of the full-flow UV disinfection within the loop.

4.1. Fish performance

The fish in the 2015_autumn batch had a significantly lower mortality (0.01%) than the spring batches (0.05; 0.06%), partly due to absence of periodic peaks in mortality. According to the RAS operators, this was normal mortality during the relevant life stages in the unit and no symptomatic fish was observed during the period. The more variable mortality of the spring batches can be related to their early stage, which is more sensitive and normally show a higher mortality than the larger fish stage produced during autumn (Tørud et al., 2019).

4.2. Dynamics of the RAS water microbiota over long time periods

In comparison with the two last batches, the first production batch (2015_spring) showed a different composition of the water microbiota, with a lower alpha diversity and more variable microbiota composition over time and between fish tanks (Figs. 4, 5, 6). This production batch was produced at the same time of the year as the last production batch in this study (2016_spring). The water microbiota of the two last production batches (2015_autumn and 2016_spring), on the other hand, were more similar in composition (Figs. 4, 5), even though the fish groups were produced at different seasons, with different size of fish, amounts of biomass, feeding regimes and several other parameters. Prior to the first production batch (2015_spring), the biofilter had been disinfected and started with clean carriers and then matured until the stocking with fish. The 2015_autumn batch had a matured biofilter that had been run continuously and without disinfection since the 2015_spring batch. Prior to the 2016_spring batch however, the complete system, including the biofilter was disinfected. The biofilter was then seeded from an already matured and running biofilter. Thus, the biofilter in the 2015_spring batch might have represented a more immature biofilm community, compared to the biofilters in the 2015_autumn and 2016_spring batches. Differences in start-up procedures of the biofilter for these three batches may explain the observed differences and similarities in the water microbiota between the production batches. Stable water microbiota over time and high alpha diversities have previously been proposed to characterize K-selected communities (Attramadal et al., 2012a, 2012b; Vadstein et al., 2018). Another implication of these observations is that the biofilm communities in the biofilters may affect the water microbiota more heavily than variables such as season, fish age, feeding routines and disinfection of the system. The knowledge on interactions between the biofilter biofilm community and the suspended bacteria in the water in RAS is limited. Some studies show that the abundance of free-living bacterial populations in the water can be correlated to the abundance of populations in the biofilm of the biofilter (Leonard et al., 2000; Michaud et al., 2014), and a selective exchange of bacteria is expected (Blancheton et al., 2013; Bartelme et al., 2017). The possibility for securing a stable and resilient microbiota in RAS, through the use of matured biofilters should be addressed in future studies.

4.3. Effects of disinfection in a RAS with a short hydraulic retention time in fish tanks

UV treatment is used as an extra hygienic barrier of the system by inactivation of potential pathogenic bacteria (Liltved et al., 1995; Sharrer et al., 2005; Hess-Erga et al., 2010). However, little is known about how efficient disinfection in the RAS loop is for preventing pathogens growing in the system. Turbid water, typical for RAS, reduce disinfection efficiency. Particles in the water are known to reduce the disinfection effect of the UV by protecting the bacteria from the UV-light (Hess-Erga et al., 2008; Huyben et al., 2018), and it can be difficult to inactivate most of the bacteria even at an excessive UV dose at high turbidity (Sharrer et al., 2005). The UV treatment kills and inactivates bacteria, but does not reduce the amount of substrate available, leading to a regrowth of opportunistic bacteria in the rearing tanks (Salvesen et al., 1999; Hess-Erga et al., 2010; Attramadal et al., 2012a, 2012b; Vadstein et al., 2018). In systems with long hydraulic retention times (HRT) in the fish tanks (60 min and longer), like marine hatcheries, significant regrowth and proliferation of opportunistic bacteria is well documented. This results in an altered microbial community composition that have negative effects on larval health and survival (Attramadal et al., 2012a; Vadstein et al., 2018; Dahle et al., 2020; Teitge et al., 2020; Attramadal et al., 2021). In the freshwater RAS for Atlantic salmon examined here, the full-flow UV treatment directly upstream the rearing tanks had no observable effect on the community composition of the water microbiota (Fig. 5). This was especially evident for the two last production batches. The similarity in community composition of the water throughout the system can be explained by the short HRT (23 min) in the fish tanks, which limits the time for regrowth in the tanks and makes the growth more likely to happen further down the line from the disinfection step, for example in the biofilter (Bakke et al., 2017; Vadstein et al., 2018). When Bray-Curtis similarities are used to compare communities, rare OTUs have little impact. Thus, rare OTUs could have been affected by the UV treatment, without effecting the Bray-Curtis similarities. However, the Sørensen-Dice index, which is based on presence – absence data, (Chao et al., 2006) and thus are more influenced by rare OTUs, did not indicate a distinct community composition through the RAS. We also used an RNA-based approach to study the active microbial response to the UV treatment. Neither this analysis indicated community changes after the UV treatment (Fig. 8). However, we did identify three *Acinetobacter* OTUs that showed a general pattern with lower abundance in samples taken downstream the UV treatment compared to those taken upstream the UV, with an average 75% reduction. This trend was especially evident for 2015_spring, which had a high abundance of *Acinetobacter* OTUs. *Acinetobacter* spp. is widespread in water ecosystems and includes both non-pathogenic, opportunistic, and fish pathogenic species (Turton et al., 2010; Hare et al., 2012). A similar strain specific reduction of *Acinetobacter* was seen by Hare et al. (2012). This indicates that *Acinetobacter* is particularly sensitive to UV treatment. Although this study included few sample events, the sampling included three production batches where all samples showed the same pattern: The full-flow UV disinfection had no observable effect on the water microbiota composition. Although immediate effects on the water microbiota were not observed, the UV disinfection may have long-term effects that influence the RAS water microbiota.

The UV treatment efficiently reduced the number of live bacteria (average 89.0% reduction of CFU), which was similar to previous studies (Huyben et al., 2018). As expected, the UV treatment efficiency was lower for more turbid water (20.1% at day 41 of 2016_spring) (Sharrer et al., 2005; Hess-Erga et al., 2008). The density of bacteria (i.e., CFU) increased 4 times in the fish tanks, indicating regrowth following the UV disinfection step. The regrowth continued through the water treatment system, reaching the highest levels of bacteria upstream of the UV treatment. The bacterial regrowth in marine hatchery rearing tanks, which have long HRT (more than one hour), can represent as much as up to a 14 time increase in bacteria numbers following disinfection, depending on the HRT (Attramadal et al., 2014; Vadstein et al., 2018). In the system studied here, with short HRT in fish tanks (23 min), the regrowth of bacteria in fish tanks was much lower (4-time increase). Moreover, the water microbiota did not change much in the fish tanks, especially for the two last production batches. This indicates that the regrowth observed in the fish tanks did not have a large impact on the

microbial community composition. It has been proposed that a fully K-selected system with a matured microbial community only can be established in RAS without UV, because point disinfection within the RAS loop will promote regrowth and selection for r-strategists in the fish tanks. Thus, UV treatment may not result in a reduction of the bacterial density of the system, but rather induce a detrimental r-selection in the fish tanks (Attramadal et al., 2012a, 2012b, 2014; Vadstein et al., 2018; Attramadal et al., 2021). The results obtained in this study indicate that these kind of negative, non-intended effects of the UV treatment is strongly reduced in RAS with short HRT in fish tanks (typically in RAS producing Atlantic salmon), and that UV disinfection can be used to restrict bacterial density without compromising the microbial water quality in the fish tanks. However, further studies should investigate the risk of successful invasion from pathogens in RAS with low HRT of tanks and UV treatment compared to systems without disinfection in the loop, as the latter is hypothesized to be more resistant to invasion.

5. Conclusions

In the RAS studied here, we found that the level similarity of the water microbiota between production batches could not be related to factors like season, fish age, and operational routines like for example feed loading. Two of the production batches had a highly similar water microbiota despite disinfection of the whole system between the batches and rearing fish of different stages. In contrast, the water microbiota of the first production batch differed from that of the others, was more variable; both through the system and over time and had a lower alpha diversity. A more immature biofilter in the first batch may explain these differences. Using a matured biofilter at start-up of a fish batch may contribute to establishing a more stable and resilient water microbiota in RAS compared to systems with newly started biofilters. Our results indicate that the biofilm communities in the biofilters may affect the water microbiota more heavily than season, fish age, feeding routines and disinfection. The UV directly upstream the rearing tanks had no observable effect on the community composition of the water microbiota. This was likely due to low hydraulic retention time (HRT) in rearing tanks, which limited the bacterial regrowth and community changes. The disinfection efficiency of the UV treatment was on average 89% when the water had low turbidity. This study shows that UV disinfection can be used to efficiently reduce bacterial density without compromising the microbial water quality surrounding the fish in RAS with low HRT in the fish tanks.

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.

Acknowledgement

We would like to thank Hege Brandsegg for practical assistance during the study and the RAS facility for providing data and allowing us to sample.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2021.737382.

References

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., Salvesen, I., Xue, R., Øie, G., Størseth, T.R., 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., Kristensen, 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.

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.

Bray, J.R, Curtis, J. T., 1957. An Ordination of the Upland Forest Communities of Wisconsin. Ecological Monographs, 27. Ecological Monographs, pp. 326–349.

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.1541-0420.2005.00489.x.

Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. Aust. J. Ecol. 18, 117–143.

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.

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.

Davis, J.C., 1986. Statistics and Data Analysis in Geology. John Wiley & Sons Inc, New York, USA.

de Jesus Gregersen, K.J., Pedersen, P.B., Pedersen, L.F., Liu, D., Dalsgaard, J., 2020. UV irradiation and micro filtration effects on micro particle development and microbial water quality in recirculation aquaculture systems. Aquaculture 518 (734785). https://doi.org/10.1016/j.aquaculture.2019.734785.

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 (1), 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.

Edgar, R.C., 2016. SINTAX: a simple non-Bayesian taxonomy classifier for 16S and ITS sequences. BioRxiv. 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., 2020a. 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., 2020b. 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.

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

Hare, J.M., Bradley, J.A., Lin, C.-L., Elam, T.J., 2012. Diverse responses to UV light exposure in Acinetobacter include the capacity for DNA damage-induced mutagenesis in the opportunistic pathogens *Acinetobacter baumannii* and *Acinetobacter ursingii*. Microbiology. 158, 601–611. https://doi.org/10.1099/mic.0.054668-0.

Hess-Erga, O.K., Attramadal, K., Vadstein, O., 2008. Biotic and abiotic particles protect marine heterotrophic bacteria during UV and ozone disinfection. Aquat. Biol. 4 (2) https://doi.org/10.3354/ab00105. ISSN 1864-7782. s147-154.

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.

Huyben, D., Bevan, D., Stevenson, R., Zhou, H., Moccia, R., 2018. Evaluation of membrane filtration and UV irradiation to control bacterial loads in recirculation aquaculture systems. Aquac. Int. 26, 1531–1540. https://doi.org/10.1007/s10499-018-0301-z.

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.

Leonard, N., Blancheton, J.P., Guiraud, J.P., 2000. Populations of heterotrophic bacteria in an experimental 483 recirculating aquaculture system. Aquac. Eng. 22, 109–120. https://doi.org/10.1016/S0144-8609(00)00035-2.

Liltved, H., Hektoen, H., Efraimsen, H., 1995. Inactivation of bacterial and viral fish pathogens by ozonation or UV irradiation in water of different salinity. Aquac. Eng. 14, 107–122.

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., 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.

Nordgård, A.S.R., Bergland, W.H., Vadstein, O., Mironov, V., Bakke, R., Østgaard, K., Bakke, I., 2017. Anaerobic digestion of pig manure supernatant at high ammonia concentrations characterized by high abundances of Methanosaeta and non-euryarchaeotal archaea. Sci. Rep. 7 (1), 1507. https://doi.org/10.1038/s41598-017-14527-1.

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

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., 2015. Microorganisms in recirculating aquaculture systems and their management. Rev. Aquac. 7 (2), 117–130. https://doi.org/10.1111/raq.12057.

Salvesen, I., Skjermo, J., Vadstein, O., 1999. Growth of turbot (*Scophthalmus maximus* L.) during first feeding in relation to the proportion of r/K-strategists in the bacterial community of the rearing water. Aquaculture. 175, 337–350. https://doi.org/10.1016/S0044-8486(99)00110-6.

Schumann, M., Brinker, A., 2020. Understanding and managing suspended solids in intensive salmonid aquaculture: a review. Rev. Aquac. 12 (4), 2109–2139. https://doi.org/10.1111/raq.12425.

Sharrer, M.J., Summerfelt, S.T., Bullock, G.L., Gleason, L.E., Taeuber, J., 2005. Inactivation of bacteria using ultraviolet irradiation in a recirculating salmonid culture system. Aquac. Eng. 33, 135–149. https://doi.org/10.1016/j.aquaeng.2004.12.001.

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.aquaeng.2008.10.002.

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. 00, 1–27. https://doi.org/10.1111/jfd.13238.

Tørud, B., Jensen, B.B., Gåsnes, S.K., Grønbech, S., Gismervik, K., 2019. Dyrevelferd i settefiskproduksjonen (SMÅVEL). Rapport 14–2019 (In Norwegian).

Turton, J.F., Shah, J., Ozongwu, C., Pike, R., 2010. Incidence of Acinetobacter species other than *A. baumannii* among clinical isolates of Acinetobacter: evidence for emerging species. J. Clin. Microbiol. 48, 1445–1449 (https://dx.doi.org/10.1128%2FJCM.02467-09).

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.

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.

Vestrum, R.I., Attramadal, K.J.K., Vadstein, O., Gundersen, M., Bakke, I., 2020. Bacterial community assembly in Atlantic cod larvae (*Gadus morhua*): contributions of ecological processes and metacommunity structure. FEMS Microbiol. Ecol. 96 https://doi.org/10.1093/femsec/fiaa163, 2020, fiaa163.