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Gregersen, Kim João de Jesus; Pedersen, Lars-Flemming; Pedersen, Per Boxbjerg; Syropoulou, Elisavet; Dalsgaard, Johanne

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Authors:
Kim João de Jesus Gregersen
Corresponding author.
Affiliation: Technical University of Denmark, DTU Aqua, Section for Aquaculture, The North Sea Research Centre, P.O. Box 101, DK-9850 Hirtshals, Denmark
jdjg@aqua.dtu.dk

Lars-Flemming Pedersen
Affiliation: Technical University of Denmark, DTU Aqua, Section for Aquaculture, The North Sea Research Centre, P.O. Box 101, DK-9850 Hirtshals, Denmark
lfp@aqua.dtu.dk

Per Bovbjerg Pedersen
Affiliation: Technical University of Denmark, DTU Aqua, Section for Aquaculture, The North Sea Research Centre, P.O. Box 101, DK-9850 Hirtshals, Denmark
pbp@aqua.dtu.dk

Elisavet Syropoulou
Affiliation: Technical University of Denmark, DTU Aqua, Section for Aquaculture, The North Sea Research Centre, P.O. Box 101, DK-9850 Hirtshals, Denmark
e.syropoulou@outlook.com

Johanne Dalsgaard
Affiliation: Technical University of Denmark, DTU Aqua, Section for Aquaculture, The North Sea Research Centre, P.O. Box 101, DK-9850 Hirtshals, Denmark
jtd@aqua.dtu.dk

Corresponding author contact information:
Email: jdjg@aqua.dtu.dk
Phone number: 0045 26228441
Address: DTU Aqua, Niels Juelsvej 30, 9850 Hirtshals, Denmark.
Abstract
Foam fractionation is often considered an ineffective way of removing organic matter from freshwater due to the low surface tension of the water. There is, however, a lack of studies testing foam fractionation efficiency in replicated freshwater recirculating aquaculture systems (RAS). Foam fractionation can be applied with or without ozone. Ozone is a strong oxidizer previously shown to improve water quality and protein skimmer efficiency. To test the efficiency of foam fractionation and ozonation (20 g O₃ kg⁻¹ feed) separately and in combination in freshwater RAS, a two-by-two factorial trial was conducted with each main factor at two levels (applied or not applied). Each treatment combination was carried out in triplicates using 12 replicated pilot scale RAS stocked with juvenile rainbow trout (Oncorhynchus mykiss) and operated at a feed loading of 1.66 kg feed m⁻³ make-up water. The trial lasted 8 weeks and samples were obtained once a week. Ozone applied by itself significantly reduced the number of particles (83 %), bacterial activity (48 %) and particulate BOD₅ (5-days biochemical oxygen demand; 54 %), and increased ultra violet transmittance (UVT; 43 %) compared to the untreated control group. Foam fractionation by itself lead to significant reductions in particle numbers and volume (58 and 62 %, respectively), turbidity (62 %), bacterial activity (54 %) and total BOD₅ (51 %).

A combination of both treatments resulted in a significant additional improvement of important water quality variables, including a 75 % reduction in total BOD₅, 79 % reduction in turbidity, 89% reduction in particle numbers and 90 % reduction in bacterial activity compared to the control.

The removal efficiencies were within the same range as those observed in previous studies conducted with foam fractionators in saltwater systems (with or without ozone), corroborating that foam fractionation may become a useful tool for controlling organic matter build-up and bacterial loads in freshwater RAS.

Keywords: Aquaculture, RAS, Foam fractionation, Ozone, Water quality, Organic matter

1. Introduction
The build-up of organic matter in recirculating aquaculture systems (RAS), deriving from fish excretions and feed spill (Schumann and Brinker, 2020), is among the largest challenges in the industry (Martins et al., 2010). Modern aquaculture facilities are typically equipped with primary solids removal technologies based on particle sedimentation (e.g. settling cones) and filtration (e.g. drum filters) (Timmons and Ebeling, 2010). As a result of prolonged retention times in RAS, together with the use of technologies which target mainly larger particles, fine solids and dissolved organic matter accumulate in the system (Chen et al., 1993a; de Jesus Gregersen et al., 2019; Fernandes et al., 2014; Patterson et al., 1999)
Accumulation of fine solids is considered problematic due to their small size and large surface area to volume ratio providing food and space for bacteria growth (Becke et al., 2020; de Jesus Gregersen et al., 2019; Pedersen et al., 2017). Similarly, dissolved nutrients and organic matter provide energy for free-living bacteria. Increased bacterial growth in RAS in turn leads to increased oxygen consumption, clogging of biofilters and potentially reducing nitrification capacity (Chen et al., 2006; Zhang et al., 1994). Organic matter build-up in stagnant areas is also thought to explain recent cases of H₂S driven mortality events (Dalsgaard, 2019; Letelier-Gordo et al., 2020).
A large portion of micro particles is composed of living microorganisms and can therefore be controlled by e.g. ultraviolet radiation (UV) (de Jesus Gregersen et al., 2020). While UV disinfection is commercially relevant due to its technological maturity and easiness of application, it does not deal with organic matter build-up which is the underlying cause of microbial growth, causing an increase in system carrying capacity (Blancheton et al., 2013; Vadstein et al., 1993).

Direct removal of fine solids can be achieved using different strategies. Reducing drum filter mesh size is one possibility but rapidly becomes costly (Dolan et al., 2013). Membrane filtration is another option shown to reduce colloidal particles in RAS by 77% and turbidity by 44% (Holan et al., 2014). However, membrane filtration is also costly and a main reason for why it is not implemented in the industry (Viadero and Noblet, 2002). Fossmark et al. (2020) for example estimated that it would increase production costs by 27% to apply membrane filtration to Atlantic salmon (Salmo salar) RAS.

An alternative technique for removing fine solids and even dissolved organic matter is foam fractionation (FF). Foam fractionation relies on surfactants in the water generating foam that removes particulate and dissolved organic matter (Timmons and Ebeling, 2010). Foam fractionation has been shown to concentrate total suspended solids (TSS) by 17 to 40 times in the foam condensate (Weeks et al., 1992), and reduce particulate matter and bacteria in saltwater RAS (Barrut et al., 2013; Brambilla et al., 2008). Recently, Ji et al. (2020) tested the combined effects of drum filters followed by FF in saltwater RAS. The results showed similar or better removal efficiency of FF compared to drum filtration when the drum filter was equipped with mesh filters of 120 and 90 µm. Only when the drum filter was equipped with a 40 µm filter did it clearly have superior removal efficiency.

Ozone (O₃) dosage can be coupled to FF. Ozone is a strong oxidizing agent that can be used directly for disinfection in RAS, if applied at sufficient concentration and contact time. Ozone addition is often followed by UV for destroying harmful ozone residuals (Gonçalves and Gagnon, 2011; Powell and Scolding, 2016). The strong oxidizing properties of O₃ allow it to break down complex molecules and reduce organic matter loads (Davidson et al., 2011; Summerfelt et al., 2009). Applying O₃ together with FF takes advantages of this property, improving foam fractionation efficiency by breaking down complex molecules so that they are more easily removed, and by increasing coalescence of particles (Li et al., 2009; Summerfelt et al., 1997) and altering bubble size distribution and surface tension (Hu and Xia, 2018; Matho et al., 2019). Another benefit of combining low doses of O₃ and FF is a reduced risk of ozone residuals (especially in freshwater) while organic matter to oxidise is present in the system (authors’ pers. obs.).

Foam fractionation has traditionally only been applied in saltwater systems due to seawaters high surface tension, whereas its efficiency in freshwater RAS is less clear. A few trials were, however, conducted nearly three decades ago. Chen et al. (1993b) conducted a study using water from fresh water RAS and treated in batch tests, showing a up concentration of total solids in the fomate, especially of organic particles smaller than 30 µm. Weeks et al. (1992) analysed the fomate produced by skimmers attached to pilot scale RAS and determined that the skimmers generated an up concentration of organic particles, total Kjeldahl nitrogen (TKN) and total...
suspended solids (TSS). However, the effects on water quality of freshwater RAS under operation still remain unknown. The objective of this study was therefore to assess the potential of FF and O₃ (separately or in combination) for improving the water quality in freshwater RAS, including effects on organic matter build-up, micro particle accumulation and bacterial activity in the water, as well as organic matter accumulation in the biofilter.

2. Materials and methods

2.1. Experimental setup

A two-by-two factorial experiment with foam fractionation and ozonation as main factors was performed in 12 replicated, 0.8 m³ pilot scale freshwater RAS (Fig. 1) at DTU Aqua in Hirtshals, Denmark. Four treatment combinations were applied: three control RAS without FF or O₃, three RAS with FF (FF); three RAS with O₃ dosing (O₃); and three RAS with FF + O₃ dosing combined (FF+O₃). Each RAS was composed of a: 100 L cylindroconical biofilter filled with 40 L RK BioElements (RK BioElements, Denmark) with a specific surface area of 750 m² m⁻³ and operated as a moving bed biofilter with an air flow of 4 L min⁻¹; a 200 L pump sump; and a 500 L cylindroconical rearing tank with a metal grid preventing fish from assessing the bottom cone, which contained a 0.8 L waste collector/settling column (Fig. 1). Two DC Runner 5.2 pumps (Aqua Medic GmbH, Bissendorf, Germany) in the pump sump pumped approximately 1500 L h⁻¹ to the biofilter and 2000 L h⁻¹ to the rearing tank, corresponding to a retention time in the rearing tank of approximately 15 min.

In order to test the effects of FF and O₃, six systems were fitted with foam fractionators (Sander Fresh Skim 200, Erwin Sander Elektroapparatebau GmbH, Germany), three systems were fitted with 1.8 m high bubble columns (same height as the FF) where O₃ was injected and the remaining three systems were kept standard as control systems. Three of the systems fitted with FF were supplied with O₃ as well (injected in the skimmer), while the remaining 3 systems were feed only air to test the effects of FF alone. Three ozone generators (Ozonizer S 500, Erwin Sander Elektroapparatebau GmbH, Germany) were used to supply O₃. Each ozoniser supplied a system fitted with a bubble column and a system fitted with a FF.

Foam fractionators were operated with a water flow rate of 1500 L h⁻¹ and an air flow rate of either 1320 L h⁻¹ (air alone) or 1200 L h⁻¹ (air) plus 120 L h⁻¹ ozonized air. Bubble columns were supplied with 120 L h⁻¹ ozonized air. Hydraulic retention time within FF and bubble columns was kept equal to ensure equal contact time in both systems. All gas intakes were controlled by flow meters (Key Instruments Variable area flow meter, Key Instruments, USA). Ozone was injected at a dosage of 20 g O₃ kg⁻¹ feed per day (83 mg O₃ h⁻¹). Incoming O₃ gas concentrations were measured using a UV spectrophotometer (at 254 nm) and flow through cell as described in Hansen et al. (2010). Furthermore, to estimate the amount of O₃ that reacted in the water, O₃ gas concentrations leaving the foam fractionators and bubble columns outflow air were measured at regular intervals.

Each system was stocked with 8.05 ± 0.03 kg juvenile rainbow trout (Oncorhynchus mykiss) of approximately 200 g each. The fish were fed a fixed amount of 100 g d⁻¹ (Efico E 920, Biomar, Denmark), and 60 L of water was...
replaced each day, resulting in a feed loading of 1.66 kg feed m⁻³. Oxygen levels were controlled using an OxyGuard Pacific system (OxyGuard International A/S, Denmark) and ranged between 85 and 90% saturation throughout the trial. Sodium bicarbonate was added when needed to keep pH between 7.0 and 7.3. Primary solids were collected in settling columns at the bottom of the tanks. Each day, the conical part of the tanks were cleaned using magnetic cleaners (Tunze care magnet, TUNZE® Aquarientechnik GmbH, Germany) and the settling columns were emptied.

The trial lasted eight weeks and samples were obtained once a week. All 12 RAS had been operated under similar conditions without foam fractionators or ozone for 13 weeks prior to the trial, feed 60 grams daily and all biofilters were fully operational. Feeding was increased from 60 to 100 grams 3 days prior to the start of the trial, and fish biomasses were weighed at the start and by the end of the trial.

2.2. Water sampling and analysis

Water samples were collected on day 0 prior to starting the foam fractionators and ozonisers. All water samples were collected in the morning before any daily routines. A 5 L water sample was collected from the sump of each RAS and split into homogeneous subsamples for individual analysis. pH was measured daily in the sump before daily routines using a Hach HQ40d Portable Multi Meter (Hach Lange, USA), and temperature was logged automatically by the OxyGuard Pacific system (OxyGuard International A/S, Denmark).

Particles between 1 and 200 µm were measured using a Multisizer 4e Coulter Counter (Beckman Coulter, Inc, Indianapolis, USA) with both a 50 µm and 280 µm aperture. Particles were grouped in size classes as described by Patterson et al. (1999). Total particle numbers (PN), total particle volume (PV) and total particle surface area (PSA) for the full range measured (1-168 µm) was calculated by summing the contribution from the different size classes.

To compare systems, particle size distributions were summarized by the β value as described by Patterson et al. (1999). In short, β value is the slope of the log-log transformed relationship between number of particles within size classes and the corresponding size class median diameter. A low β value indicates a system dominated by larger particles whereas a high β value indicates a system dominated by smaller particles.

Turbidity was measured using a Hach 2100Q (Hach Lange, USA), while UVT was measured using a UV spectrophotometer (Beckman DU® 530 Life Science UV/Vis Spectrophotometer, Bechman Coulter Inc, Indianapolis, USA) measuring % transmission in quartz cuvettes at 254 nm. Microbial activity in the water was quantified using a hydrogen peroxide (H₂O₂) decomposition rate assay described in Pedersen et al. (2019), considering the degradation rate constant (k, h⁻¹) as an expression of microbial activity. In short, a 42 ml water sample was placed in a 50 ml centrifuge tube and H₂O₂ was added at a final concentration of 10 mg L⁻¹. The decomposition of H₂O₂ was subsequently measured by collecting samples before addition of H₂O₂ (background level), immediately after H₂O₂ addition and every 15 min thereafter for 1 hour. The samples were kept in a water bath at 22°C for the duration of the assay. The degradation rate constant (k, h⁻¹) was calculated using the data.
obtained. Additionally, microbial activity was measured using the BactiQuant (Mycometer A/S, Denmark) assay, expressing microbial activity as relative BQ values.

Organic matter was measured as the 5-days biological oxygen demand (BOD$_5$) and chemical oxygen demand (COD). Both metrics were measured in raw, non-filtered (BOD$_{5\-Tot}$ and COD$_{Tot}$) and filtered (BOD$_{5\-Diss}$ and COD$_{Diss}$) water samples using 0.45 µm filters (Advantec® membrane filter, Toyo Roshi Kaisha Ltd, Japan). Corresponding particulate fractions (BOD$_{5\-Part}$ and COD$_{Part}$) were calculated as the difference between the non-filtered and the filtered sample. BOD$_5$ was measured following ISO 5815 (1989) modified by adding allylthiourea (ATU) (Fluka Chemika), while COD was measured following ISO 6060 (1989). Nitrate-N, nitrite-N and ammonium-N were measured by spectrophotometry following ISO 7890-1 (1986), DS 223 (1991) and DS 224 (1975), respectively.

Eight bio-elements from each biofilter were collected weekly and placed dry in 50 ml test tubes that were stored at -20 ºC prior to COD analysis. To detach the organic matter, 20 ml Milli-Q water was added to each test tube and the tubes sonicated for 10 min using a Bransonic® ultrasonic cleaner (Branson Ultrasonics Corp, USA). The resulting water was transferred to a beaker and analysed for COD$_{Tot}$ as described above. Ozone concentrations in the water were measured using the colorimetric N,N-diethyl-p-phenylenediamine (DPD) method (Buchan et al., 2005; Schroeder et al., 2015) and the indigo method (Ozone AccuVac® Ampules, Hach Lange, USA).

### 2.3. Data analysis

All data are presented as average ± standard deviation. Statistical analyses were performed in SigmaPlot 13.0 (Systat software Inc., USA). Results of the two main factors (i.e., foam fractionation and ozonation) were compared using data from the last three trial weeks (n = 9) to account for system weekly variability. Data were tested for normality (Shapiro-Wilk test) and equal variance (Brown-Forsythe). Data that did not meet these requirements were log transformed. A two-way ANOVA analysis followed by a Holm-Sidak analysis was conducted in case of significant main effects. Differences were considered significant at p < 0.05. As BactiQuant and BOD$_{5\-Diss}$ results did not meet the equal variance assumption either before or after conversion they were not subjected to two-way ANOVA analysis. Removal percentages were calculated relative to the control treatment based on averages of the last three trial weeks as: \(\text{% removal} = \frac{\text{Treatment} - \text{Control}}{\text{Control}} \times 100\).
levels by the end of the trial, while nitrite was significantly lower in systems fitted with foam fractionators (Tab. 1).

3.1. Micro particles
Micro particle numbers declined in the first half of the trial, including control systems (Fig. 2a). Systems treated with ozone displayed rapid declines within the first week (over 80 % reduction in numbers) and remained stable at a low level until the end of the trial. Systems fitted with foam fractionators showed a much slower reduction in numbers, resulting in a final reduction of 58 % compared to the control. Both factors combined resulted in significantly lower particle numbers in the water.

Particle volumes increased in the control systems and in systems with ozone only, during the trial, albeit at different rates (Fig. 2b). Systems fitted with foam fractionators declined in the start and remained stable at low levels. By the end of the trial, both the use of O₃ and FF had led to significant reductions in particle volume compared to the control. Ozonisers alone resulted in a 32 % reduction, foam fractionators reduced particle volume by 62 % and the combination of both treatments resulted in a 75 % reduction compared to the control.

As with the previous two metrics, particle surface area was also affected by the two treatments, while it remained stable at 30.4 ± 8.8 mm² ml⁻¹ in the control group (Tab. 2). Foam fractionation resulted in a 53 % reduction of total surface area, O₃ treatment in a 68 % reduction and a combination of both treatments resulted in a 83 % reduction of particle surface area compared to the control. Beta values were only affected by the use of ozone. Control systems and systems with foam fractionators had similar β values by the end of the trial (3.74 and 3.77 respectively), while systems treated with O₃ displayed significantly lower β values (3.17 and 3.24 for O₃ and FF+O₃ treatments, respectively).

3.2. Microbial activity
Bacterial activity, measured with the H₂O₂ degradation rate assay, was significantly affected by the two treatment methods (Fig. 2c). Activity declined particularly rapidly in systems treated with ozone, with activity after one week being reduced by 91 % in systems with ozonisers only and 96 % in systems with FF+O₃ treatments compared to the control. However, activity in systems treated with ozone only appeared to increase again and by the end of the trial were 48 % lower than the control. Bacterial activity in systems with foam fractionators was reduced by 61 %, while activity in systems with both ozonisers and foam fractionators remained low (90 % reduction) compared to the control. Bacterial activity measured using the BactiQuant assay closely followed the H₂O₂ degradation rate constants except that bacterial activity in O₃ treated systems was almost similar to the control by the end of the trial (Tab. 1). Due to the lack of equal variance of the BactiQuant values, data were not subjected to a statistical analysis.
3.3. Turbidity and UV transmittance (UVT)

Turbidity was significantly improved by both foam fractionation and ozonation (Tab. 2). By the end of the trial, a 65 % improvement in turbidity was achieved by foam fractionation compared to the control and 79 % when combining both treatments. Ozonation by itself resulted in a 38 % improvement by the end compared to the control group. However, as for bacteria activity, turbidity appeared to increase after an initial drop when applying ozone by itself.

Foam fractionation without ozone resulted in a 15 % improvement in UVT, while direct ozone or in combination with foam fractionation resulted in 43 and 47 % improvement, respectively. Ultraviolet transmittance was the only measurement where there was interaction between treatments (Tab. 2), and it was therefore not possible to conclude about main effects.

3.4. BOD₅

Total BOD₅ was significantly affected by both foam fractionation and ozonation resulting in reductions of 51 %, 43 % and 75 % for FF, O₃ and FF+O₃, respectively compared to the control (Fig. 2d). The development in BOD₅-Part was similar with that of BOD₅-Tot for all treatment combinations (Tab. 2). By the end of the trial, foam fractionation alone and direct ozonation had led to similar reductions in BOD₅-Part compared to control of 56 and 54 %, respectively, while a combination of the two resulted in an 84 % reduction. In contrast to total and particulate BOD₅, the different treatments seemed to have little effect on BOD₅-Diss (Tab. 2). Lack of equal variance, however, meant that no statistical analysis was performed.

3.5. COD – Water and biofilter

COD was only measured in the last 3 weeks to access final values, so no considerations are made regarding trends.

COD₅-Tot was significantly affected by both foam fractionation and ozonation with a combination of the two resulting in the largest decrease compared to the control (58 % reduction). Foam fractionation and ozonation by themselves resulted in similar reductions of 39 and 33 %, respectively. Both treatment types affected COD₅-Part significantly, with reductions of 69, 36 and 80%, respectively in systems with either foam fractionation, ozonation or a combination of the two (Tab. 2). Dissolved COD was also significantly affected by the different treatments. As with every other metric, the combination of foam fractionation and ozonation had the largest effect reducing COD₅-Diss by 40 %. Foam fractionation by itself reduced COD₅-Diss by 16 %, while ozonation reduced it by 31 %.

Although all treatments seemingly lowered COD₅-Tot levels in the biofilters compared to the control group, there were no significant differences (p > 0.05) by the end of the trial in total COD in the biofilter elements (approximately 17 % lower value in systems with ozonation only, and 23 % lower values in systems with foam fractionation).
4. Discussion

The different treatments had clear visual effects on the water colour and transparency as seen in Figure 3. The systems fitted with ozone lost most of the “yellow” colour, while the overall turbidity was reduced in system fitted with FF. The loss of yellow colour was likely caused by oxidation of humic substances as seen in previous studies (Davidson et al., 2011; Schroeder et al., 2011; Spiliotopoulou et al., 2018).

The systems were operated for 13 weeks prior to the start of the trial with a lower feed loading (1 kg m$^{-3}$). This was changed a few days prior to the start of the trial when daily feed allocation was increased from 60 to 100 g d$^{-1}$. It is likely that this change resulted in the increase of some of the metrics, which could explain some of the initial variation in the control group (initial increase in numbers followed by a re-stabilization).

4.1. Foam fractionation

Foam fractionation has been shown to reduce organic matter loads in RAS (Barrut et al., 2013; Brambilla et al., 2008; Ji et al., 2020; Weeks et al., 1992). Most of the previous studies were conducted in saltwater as foam fractionation is anticipated to have minimal effect in freshwater RAS due to lower surface tension (Timmons and Ebeling, 2010). However, the current trial showed that foam fractionation also works well in freshwater with positive effects on all measured metrics. The positive impact of foam fractionation appeared to manifest at a slower pace than that of direct ozonation, with a steady removal of organic matter over the course of 3 to 4 weeks (Fig. 2). The foam fractionator seemed particularly effective at controlling particulate organic loads and particle volume (both BOD$_{b-Part}$ and COD$_{Part}$). These results are similar to those obtained by Barrut et al. (2013) using a vacuum airlift foam fractionator in seawater RAS and obtaining an approximate 80% removal of particulate organic matter measured as dry matter. Brambilla et al. (2008), testing foam fractionation for removing organic matter and heterotrophic bacteria from seawater RAS with seabass (Dicentrarchus labrax), obtained removal rates of total suspend solids (TSS) between 12 and 40% over a single pass. The treatment in that study affected both the smallest (0.22 - 1.22 µm) and largest (> 60 µm) size fractions measured. In the current study, a uniform β value suggests that particles of all sizes were affected.

Bacterial activity was also strongly affected by foam fractionation. The approximately 60% reduction obtained in the current trial is similar to that obtained by Brambilla et al. (2008) in a seawater RAS, achieving 55 - 90% removal depending on operational conditions, using count of viable heterotrophic bacteria in agar plates. Likewise, Rahman et al. (2012) achieved 2.6 times lower bacterial levels compared to a control in seawater hybrid abalone (Haliotis discus hannai X H. sieboldii) pilot scale RAS fitted with foam fractionation. In the current study, a lower level of nitrite was found in the systems fitted with foam fractionators. A possible explanation for this could be an improved biofiltration process, resulting from a reduced competition from heterotrophic bacteria caused by lower levels of organic matter present in the system (Zhang et al., 1994).

The simultaneous reduction in both organic matter and bacterial activity observed in the current study suggests a direct removal of bacteria by foam fractionation in freshwater, similarly to that observed in seawater. In addition,
the reduction in organic matter reduces a systems overall carrying capacity (Vadstein et al., 1993) making it less prone to potentially harmful bacteria blooms.

4.2. Ozone

Unlike systems fitted with only foam fractionators, which showed progressive reduction in all metrics in the first half of the trial, systems dosed with ozone showed immediate responses and most metrics reached their lowest levels within the first few weeks. This development was most likely a result of ozone’s oxidizing effect on bacteria and a resulting self-perpetuating pattern leading to a cumulative improvement in water quality, as corroborated by the rapid decline in bacterial activity and particle numbers compared to the control. Part of the effect was also likely caused by improved solids removal as ozone is known to improve solids removal efficiency. Park et al. (2013) for example found that ozone improved solids removal in a radial flow settler, while Summerfelt et al. (1997) found that ozone improved microscreen filtration. It is likely that ozone had similar effects in the current trial as the reduction in particle volume, BOD\textsubscript{5}Part and COD\textsubscript{Part} was similar to that observed in previous trials (Good et al., 2011; Park et al., 2013; Rueter and Johnson, 1995; Summerfelt et al., 1997). Examining the effects of ozone by itself in replicated RAS, Davidson \textit{et al.} (2011) found that ozonation lead to a reduction in BOD\textsubscript{5}, total organic carbon (TOC), dissolved organic carbon (DOC), TSS, and heterotrophic bacteria abundance, while UVT increased. Systems treated with ozonation only displayed an increase over time in most metrics. We speculate that this increase was caused by a too low realised O\textsubscript{3} dose. While a nominal dose of 20 g O\textsubscript{3} kg\textsuperscript{-1} feed was applied, measurements of air leaving the treatment units suggested that an ozone transfer rate to the water of approximately 35 % was achieved (in both the bubble columns and FF), corresponding to an actual dose of about 7 g kg\textsuperscript{-1} feed. It is possible that this lower dose allowed bacteria with higher O\textsubscript{3} tolerance to proliferate. The hypothesis is supported by the observed increase in bacterial activity accompanied by a similar increase in particle volume, suggesting that bacteria were aggregating. At the same time, particle numbers did not increase suggesting that free swimming bacteria were removed or eliminated. Bacteria in biofilms and bacteria associated with particles are generally more resistant to disinfection, including O\textsubscript{3}, than free living bacteria (Hess-Erga \textit{et al.}, 2008).

One of the issues arising when using ozone in a system is its potential toxicity to the fish (Gonçalves and Gagnon, 2011; Powell and Scolding, 2016; Stiller et al., 2020). This risk seems minimal in the current study as no ozone was detected in the water measured both via the DPD and indigo method. Ozone presumably reacted immediately with the organic matter available as seen in a previous study on the combined use of O\textsubscript{3} and foam fractionators (Guilherme \textit{et al.}, 2020).

4.3. Combined effects

Combining foam fractionation with ozonation lead to additional improvements in the water quality parameters. Ozone is typically applied together with foam fractionation (Attramadal \textit{et al.}, 2012; Park \textit{et al.}, 2011, 2013;
Schroeder et al., 2011) as it is an efficient way of transferring ozone. Similarly, ozone improves foam fractionation removal efficiency by degrading complex molecules and improving particle flocculation by formation of smaller bubbles (Li et al., 2009; Rueter and Johnson, 1995). In the current study, ozone primary affected micro particle numbers and UVT presumably by killing free swimming bacteria (resulting in a decline in particle numbers) and oxidizing dissolved substances (e.g. humic substances) that would otherwise absorb and refract light. On the other hand, by removing solids foam fractionation led to a reduction in particulate volume, particulate COD and turbidity. Combined, this presumably led to a reduction in system carrying capacity, aggravating the conditions for bacterial growth. Furthermore, the combined use of foam fractionation and ozonation may potentially reduce the risk of unwanted biofilm formation and reduce the consumption of oxygen by heterotrophic bacteria degrading organic matter.

4.4. Effects on biofilters

Few studies have addressed the potential implications of different treatments on biofilters in RAS and their role in storing and releasing organic matter (de Oliveira et al., 2019). As discussed in a previous study (de Jesus Gregersen et al., 2020), a decline in organic matter in the water might be accompanied by translocation of organic matter to the biofilter. To resolve this, the current study examined the organic matter (total COD) associated with biofilter elements. Although not significant, a lower organic matter build-up was observed in all treated systems compared to the control, suggesting that the applied treatments not only improved water quality directly but also overall “system quality”. This is further supported by a lower level of nitrite in systems treated with foam fractionation. A mass balance analysis of the organic matter present in the system (measured as COD\textsubscript{total}) showed that despite only making up 5% of the total volume of the system and being operated as a moving bed biofilter, the bioelements in the biofilter contained between 23 and 36% of all organic matter present in the system (depending on treatment), reinforcing the need to better understand organic matter processes within biofilters.

5. Summary and future perspectives

The current study provided new knowledge about the effects of foam fractionation and ozone on the water quality in freshwater RAS, and demonstrated the potential benefits on biofiltration by reducing the amount of organic matter in the system. The study demonstrated that using foam fractionation in freshwater RAS may lead to similar reductions in organic matter as that observed in saltwater RAS. Furthermore, the study confirmed the positive effects of ozone on overall RAS water quality. Organic matter removal efficiency from foam fractionation was further improved by simultaneous application of ozone. While ozone is already used in both freshwater and saltwater RAS and foam fractionation is used in saltwater RAS, foam fractionation is to our best knowledge not yet applied in commercial freshwater RAS. As demonstrated here, foam fractionation may have large potentials in freshwater RAS as well, either by itself or in combination with ozone for improving rearing conditions and maintaining high water quality standards. The large reductions in organic matter in the systems, accompanied by
a reduced level of bacterial activity and an apparent increase in biofilter nitrification efficiency, can lead to a decrease in the use of disinfectants as well as an improvement in overall production quality. To resolve the most optimal use of foam fractionation in freshwater RAS and make specific recommendations to the industry on best application of the technology, supplementary studies of fish performance are needed.

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References

Attramadal, K.J.K., Øie, G., Størseth, T.R., Alver, M.O., Vadstein, O., Olsen, Y., 2012. 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

Barrut, B., Blancheton, J.-P., Callier, M., Champagne, J.-Y., Grasmick, A., 2013. Foam fractionation efficiency of a vacuum airlift-Application to particulate matter removal in recirculating systems. Aquac. Eng. 54, 16–21.

Becke, C., Schumann, M., Geist, J., Brinker, A., 2020. Shape characteristics of suspended solids and implications in different salmonid aquaculture production systems. Aquaculture 516, 734631.

https://doi.org/10.1016/j.aquaculture.2019.734631

Blancheton, J.P., Attramadal, K.J.K., Michaud, L., d’Orbacastel, E.R., Vadstein, O., 2013. Insight into bacterial population in aquaculture systems and its implication. Aquac. Eng. 53, 30–39.

https://doi.org/10.1016/j.aquaeng.2012.11.009

Brambilla, F., Antonini, M., Ceccuzzi, P., Terova, G., Saroglia, M., 2008. Foam fractionation efficiency in particulate matter and heterotrophic bacteria removal from a recirculating seabass (Dicentrarchus labrax) system. Aquac. Eng. 39, 37–42.

Buchan, K.A.H., Martin-Robichaud, D.J., Benfey, T.J., 2005. Measurement of dissolved ozone in sea water: A comparison of methods. Aquac. Eng. 33, 225–231.

Chen, S., Ling, J., Blancheton, J.P., 2006. Nitrification kinetics of biofilm as affected by water quality factors. Aquac. Eng. 34, 179–197. https://doi.org/10.1016/j.aquaeng.2005.09.004

Chen, S., Timmons, M.B., Aneshansley, D.J., Bisogni, J.J., 1993a. Suspended solids characteristics from recirculating aquacultural systems and design implications. Aquaculture 112, 143–155.
Chen, S., Timmons, M.B., Bisogni, J.J., Aneshansley, D.J., 1993b. Suspended-Solids Removal by Foam Fractionation. Progress. Fish-Cultural 55, 69–75.

Dalsgaard, J., 2019. 5th NordicRAS Workshop on Recirculating Aquaculture Systems. Berlin, Germany, 7-8 October 2019. Book of Abstracts.

Davidson, J., Good, C., Welsh, C., Summerfelt, S., 2011. The effects of ozone and water exchange rates on water quality and rainbow trout Oncorhynchus mykiss performance in replicated water recirculating systems. Aquac. Eng. 44, 80–96. https://doi.org/10.1016/j.aquaeng.2011.04.001

de Jesus Gregersen, K.J., Pedersen, P.B., Pedersen, L.F., Dalsgaard, J., 2019. Micro particles and microbial activity in Danish recirculating rainbow trout (Oncorhynchus mykiss) farms. Aquac. Eng. 84, 60–66. https://doi.org/10.1016/j.aquaeng.2018.12.001

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

de Oliveira, F.F., Moreira, R.G., Schneider, R.P., 2019. Evidence of improved water quality and biofilm control by slow sand filters in aquaculture – A case study. Aquac. Eng. 85, 80–89. https://doi.org/10.1016/j.aquaeng.2019.03.003

Dolan, E., Murphy, N., O’Hehir, M., 2013. Factors influencing optimal micro-screen drum filter selection for recirculating aquaculture systems. Aquac. Eng. 56, 42–50. https://doi.org/10.1016/j.aquaeng.2013.04.005

DS 223, 1991. Water analysis - determination of the sum of nitrite- and nitrate-nitrogen. Danish Standards Foundation, Charlottenlund, Denmark

DS 224, 1975. Water analysis - determination of ammonia-nitrogen. Danish Standards Foundation, Charlottenlund, Denmark

Fernandes, P.M., Pedersen, L.F., Pedersen, P.B., 2014. Daily micro particle distribution of an experimental recirculating aquaculture system-A case study. Aquac. Eng. 60, 28–34. https://doi.org/10.1016/j.aquaeng.2014.03.007

Fossmark, R.O., Vadstein, O., Rosten, T.W., Bakke, I., Košeto, D., Bugten, A. V., Helberg, G.A., Nesje, J., Jørgensen, N.O.G., Raspati, G., Azrage, 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

Gonçalves, A.A., Gagnon, G.A., 2011. Ozone Application in Recirculating Aquaculture System: An Overview. Ozone Sci. Eng. 33, 345–367.

Good, C., Davidson, J., Welsh, C., Snekvik, K., Summerfelt, S., 2011. The effects of ozonation on performance, health and welfare of rainbow trout Oncorhynchus mykiss in low-exchange water recirculation aquaculture
Guilherme, M.F., de Jesus Gregersen, K.J., Pedersen, L.F., 2020. Effects of foam fractionation and chemical disinfection on the removal of different microalgae cultures. Aquac. Res. 1–10. https://doi.org/10.1111/are.14663

Hansen, K.M.S., Andersen, H.R., Ledin, A., 2010. Ozonation of estrogenic chemicals in biologically treated sewage. Water Sci. Technol. 62, 649–657. https://doi.org/10.2166/wst.2010.919

Hess-Erga, O.K., Attramadal, K.J.K., Vadstein, O., 2008. Biotic and abiotic particles protect marine heterotrophic bacteria during UV and ozone disinfection. Aquat. Biol. 4, 147–154. https://doi.org/10.3354/ab00105

Holan, A.B., Wold, P.A., Leiknes, T.O., 2014. Intensive rearing of cod larvae (Gadus morhua) in recirculating aquaculture systems (RAS) implementing a membrane bioreactor (MBR) for enhanced colloidal particle and fine suspended solids removal. Aquac. Eng. 58, 52–58. https://doi.org/10.1016/j.aquaeng.2013.10.001

Hu, L., Xia, Z., 2018. Application of ozone micro-nano-bubbles to groundwater remediation. J. Hazard. Mater. 342, 446–453. https://doi.org/10.1016/j.jhazmat.2017.08.030

ISO 5815, 1989. Water quality – Determination of biochemical oxygen demand after n days (BODn) – Part 2: Method for undiluted samples. International Organization for Standardization, Geneva, Switzerland

ISO 6060, 1989. Water quality – Determination of the chemical oxygen demand. International Organization for Standardization, Geneva, Switzerland

Ji, M., Li, H., Li, J., Ye, Z., Zhu, S., 2020. Effect of Mesh Size on Microscreen Filtration Combined with Foam Fractionation for Solids Removal in Recirculating Aquacultural Seawater. N. Am. J. Aquac. 82, 215–223. https://doi.org/10.1002/naaq.10147

Letelier-Gordo, C.O., Aalto, S.L., Suurnäkki, S., Pedersen, P.B., 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

Li, T., Yan, X., Wang, D., Wang, F., 2009. Impact of preozonation on the performance of coagulated flocs. Chemosphere 75, 187–192. https://doi.org/10.1016/j.chemosphere.2008.12.014

Martins, C.I.M., Eding, E.H., Verdegem, M.C.J., Heinsbroek, L.T.N., Schneider, O., Blancheton, J.P., Roque d’Orbcastel, E., Verreth, J.A.J., 2010. New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. Aquac. Eng. 43, 83–93.

Matho, C., Schwarzenberger, K., Eckert, K., Keshavarzi, B., Walther, T., Steingroewer, J., Krujatz, F., 2019. Bio-compatible flotation of Chlorella vulgaris: Study of zeta potential and flotation efficiency. Algal Res. 44, 101705. https://doi.org/10.1016/j.algal.2019.101705

Park, J., Kim, P.K., Lim, T., Daniels, H. V., 2013. Ozonation in seawater recirculating systems for black seabream Acanthopagrus schlegelii (Bleeker): Effects on solids, bacteria, water clarity, and color. Aquac. Eng. 55, 1–8. https://doi.org/10.1016/j.aquaeng.2013.01.002

Park, J., Kim, Y., Kim, P.K., Daniels, H. V., 2011. Effects of two different ozone doses on seawater recirculating
systems for black sea bream Acanthopagrus schlegeli (Bleeker): Removal of solids and bacteria by foam fractionation. Aquac. Eng. 44, 19–24. https://doi.org/10.1016/j.aquaeng.2010.11.001

Patterson, R.N., Watts, K.C., Timmons, M.B., 1999. The power law in particle size analysis for aquacultural facilities. Aquac. Eng. 19, 259–273. https://doi.org/10.1016/S0144-8609(98)00054-5

Pedersen, L.F., Rojas-Tirado, P., Arvin, E., Pedersen, P.B., 2019. Assessment of microbial activity in water based on hydrogen peroxide decomposition rates. Aquac. Eng. 85, 9–14. https://doi.org/10.1016/j.aquaeng.2019.01.001

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

Powell, A., Scolding, J.W.S., 2016. Direct application of ozone in aquaculture systems. Rev. Aquac. 10, 424–438. https://doi.org/10.1111/raq.12169

Rahman, M.M., Kadowaki, S., Linn, S.M., Yamada, Y., 2012. Effects of protein skimming on water quality, bacterial abundance and abalone growth in land based recirculating aquaculture systems. J. Fish. Aquat. Sci. 7, 150–161. https://doi.org/10.3923/jfas.2012.150.161

Rueter, J., Johnson, R., 1995. The use of ozone to improve solids removal during disinfection. Aquac. Eng. 14, 123–141. https://doi.org/10.1016/0144-8609(94)P4431-A

Schroeder, J.P., Croot, P.L., Von Dewitz, B., Waller, U., Hanel, R., 2011. Potential and limitations of ozone for the removal of ammonia, nitrite, and yellow substances in marine recirculating aquaculture systems. Aquac. Eng. 45, 35–41. https://doi.org/10.1016/j.aquaeng.2011.06.001

Schroeder, J.P., Klatt, S.F., Schlachter, M., Zablotski, Y., Keuter, S., Spieck, E., Schulz, C., 2015. Impact of ozonation and residual ozone-produced oxidants on the nitrification performance of moving-bed biofilters from marine recirculating aquaculture systems. Aquac. Eng. 65, 27–36. https://doi.org/10.1016/j.aquaeng.2014.10.008

Schumann, M., Brinker, A., 2020. Understanding and managing suspended solids in intensive salmonid aquaculture: a review. Rev. Aquac. 1–31. https://doi.org/10.1111/raq.12425

Spiliotopoulou, A., Rojas-Tirado, P., Chhetri, R.K., Kaarsholm, K.M.S., Martin, R., Pedersen, P.B., Pedersen, L.F., Andersen, H.R., 2018. Ozonation control and effects of ozone on water quality in recirculating aquaculture systems. Water Res. 133, 289–298. https://doi.org/10.1016/j.watres.2018.01.032

Stillier, K.T., Kolarevic, J., Lazado, C.C., Gerwins, J., Good, C., Summerfelt, S.T., Mota, V.C., Espmark, Å.M.O., 2020. The effects of ozone on Atlantic salmon post-smolt in brackish water—establishing welfare indicators and thresholds. Int. J. Mol. Sci. 21, 1–17. https://doi.org/10.3390/ijms21145109

Summerfelt, S.T., Hankins, J.A., Weber, A.L., Durant, M.D., 1997. Ozonation of a recirculating rainbow trout culture system. II. Effects on microscreen filtration and water quality. Aquaculture 158, 57–67. https://doi.org/10.1016/S0044-8486(97)00064-1

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, 17–27. https://doi.org/10.1016/j.aquaeng.2008.10.002

Timmons, M.B., Ebeling, J.M., 2010. Recirculating Aquaculture, 2nd ed. Cayuga Aqua Ventures, NY, USA.

Vadstein, O., Øie, G., Salvesen, I., Skjermo, J., 1993. A strategy to obtain microbial control during larval development of marine fish. Fish Farming Technol. 69–75.

Viadero, R.C., Noblet, J.A., 2002. Membrane filtration for removal of fine solids from aquaculture process water. Aquac. Eng. 26, 151–169. https://doi.org/10.1016/S0144-8609(02)00011-0

Weeks, N.C., Timmons, M.B., Chen, S., 1992. Feasibility of using foam fractionation for the removal of dissolved and suspended solids from fish culture water. Aquac. Eng. 11, 251–265. https://doi.org/10.1016/0144-8609(92)90008-L

Zhang, T.C., Fu, Y.C., Bishop, P.L., 1994. Competition in Biofilms. Water Sci. Technol. 29, 263–270.

Table 1. Average water and biofilter results of the 3 last weeks of sampling (± standard deviation). * indicates statistical significant effects of the main factors (FF and O₃), while † indicates interactions between main factors.

| Treatment       | Control    | Foam fractionator | Ozone     | Foam fractionator + Ozone | Units          |
|-----------------|------------|-------------------|-----------|---------------------------|----------------|
| Num. Particles  | 2.43 ± 1.38| 1.01 ± 1.01*      | 0.42 ± 0.22* | 0.27 ± 0.14             | million ml⁻¹  |
| Vol. Particles  | 0.037 ± 0.012| 0.014 ± 0.003*    | 0.025 ± 0.006* | 0.009 ± 0.002          | mm³ ml⁻¹      |
| S. A. particles | 30.39 ± 8.77| 14.32 ± 5.75*     | 9.84 ± 2.52* | 5.23 ± 1.95             | mm² ml⁻¹      |
| β value         | 3.74 ± 0.24| 3.77 ± 0.28       | 3.20 ± 0.22* | 3.28 ± 0.26             | dimensionless |
| Turbidity       | 7.02 ± 2.56| 2.46 ± 0.83*      | 4.34 ± 1.07* | 1.49 ± 0.43             | NTU           |
| UVT             | 51.72 ± 2.59| 59.37 ± 2.01*    | 73.75 ± 4.48²| 75.94 ± 1.36           | % transmission|
| H₂O₂            | 0.84 ± 0.24| 0.33 ± 0.17*      | 0.44 ± 0.27* | 0.08 ± 0.03             | k¹            |
| Bactiquant      | 77011 ± 32480| 35779 ± 24185    | 65674 ± 30563| 17110 ± 6172           | BQV           |
| BOD₅Total       | 6.09 ± 1.05| 2.99 ± 0.89*      | 3.45 ± 0.55* | 1.53 ± 0.24             | mg O₂ l⁻¹     |
| BOD₅Dissol      | 0.82 ± 0.13| 0.67 ± 0.10       | 1.01 ± 0.33 | 0.67 ± 0.04             | mg l⁻¹        |
| BOD₅Part        | 5.27 ± 0.98| 2.33 ± 0.88*      | 2.44 ± 0.69* | 0.86 ± 0.023            | mg l⁻¹        |
| COD₅Total       | 37.64 ± 5.86| 22.84 ± 2.70*    | 25.21 ± 2.90*| 16.01 ± 1.49           | mg l⁻¹        |
| COD₅Dissol      | 21.36 ± 1.71| 17.84 ± 1.01*    | 14.83 ± 1.05*| 12.78 ± 0.78           | mg l⁻¹        |
| COD₅Part        | 16.29 ± 4.74| 5.00 ± 2.91*     | 10.39 ± 2.93*| 3.23 ± 1.94             | mg l⁻¹        |
| Ammonium        | 74.7 ± 30.0| 83.8 ± 17.9       | 88.5 ± 36.7 | 82.9 ± 11.6             | µg NH₄-N l⁻¹  |
| Nitrite         | 119.3 ± 24.5| 77.5 ± 20.6*     | 104.0 ± 24.3| 70.5 ± 24.26           | µg NO₂-N l⁻¹  |
| Nitrate         | 57.5 ± 2.57| 56.7 ± 2.70       | 57.4 ± 2.33 | 56.6 ± 2.65             | mg NO₃-N l⁻¹  |
| Biofilter COD   | 9.3 ± 2.2 | 7.2 ± 2.4         | 7.5 ± 1.9  | 7.2 ± 1.0              | g             |
Table 2. Statistical results of the two-way analysis of variance (ANOVA).

| Treatment         | Within FF |       | Within O$_3$ |       | Interactions |       |
|-------------------|-----------|-------|---------------|-------|--------------|-------|
|                   | F         | P     | F             | P     | F           | P     |
| Num. Particles    | 8.25      | 0.007 | 41.2          | <0.001| 0.8         | 3.660 |
| Vol. Particles    | 118.5     | <0.001| 19.1          | <0.001| 0.03        | 0.867 |
| S. A. particles   | 34.4      | <0.001| 72.2          | <0.001| 0.2         | 0.625 |
| β value           | 0.4       | 0.524 | 33.1          | <0.001| 0.06        | 0.803 |
| Turbidity         | 89.5      | <0.001| 17.3          | <0.001| 0.03        | 0.875 |
| UVT               | 23.9      | <0.001| 367.8         | <0.001| 7.4         | 0.011 |
| H$_2$O$_2$        | 37.0      | <0.001| 21.4          | <0.001| 1189        | 0.284 |
| Bactiquant*       | -         | -     | -             | -     | -           | -     |
| BOD$_5$-Tot       | 107.1     | <0.001| 65.0          | <0.001| 0.2         | 0.635 |
| BOD$_5$-Diss*     | -         | -     | -             | -     | -           | -     |
| BOD$_5$-Part      | 77.8      | <0.001| 56.8          | <0.001| 0.5         | 0.471 |
| COD$_{Tot}$       | 114.1     | <0.001| 71.5          | <0.001| 0.2         | 0.630 |
| COD$_{Diss}$      | 44.2      | <0.001| 202.9         | <0.001| 0.37        | 0.545 |
| COD$_{Part}$      | 60.4      | <0.001| 10.4          | 0.003 | 3.0         | 0.091 |
| Ammonium          | 1.7       | 0.203 | 0.12          | 0.731 | 1.9         | 0.173 |
| Nitrite           | 39.1      | <0.001| 1.4           | 0.251 | 0.01        | 0.911 |
| Nitrate           | 0.8       | 0.387 | 0.03          | 0.864 | 0.0007      | 0.980 |
| Biofilter COD     | 0.06      | 3.832 | 1.1           | 0.297 | 1.2         | 0.286 |

*Statistical analysis not possible due to non-equal variance
Figure 1. Pilot scale RAS including a: 1) rearing tank; 2) moving bed biofilter; 3) pump sump; and 4) sludge collector.

Figure 2. Variation in selected water quality parameters during the trial. a) Number of particles b) volume of particles c) microbial activity (H$_2$O$_2$ degradation) d) BOD$_{STOT}$. Statistically significant effects are reported in table 2.
Figure 3. Visible effects of the different treatments on water clarity. From left to right: Ozone, Ozone + foam fractionator, control and finally foam fractionator.

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: