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6th NordicRAS Workshop on Recirculating Aquaculture Systems, Tromsø, Norway, 3-4 October 2023

Book of Abstracts

Dalsgaard, Johanne

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By Johanne Dalsgaard (ed.)

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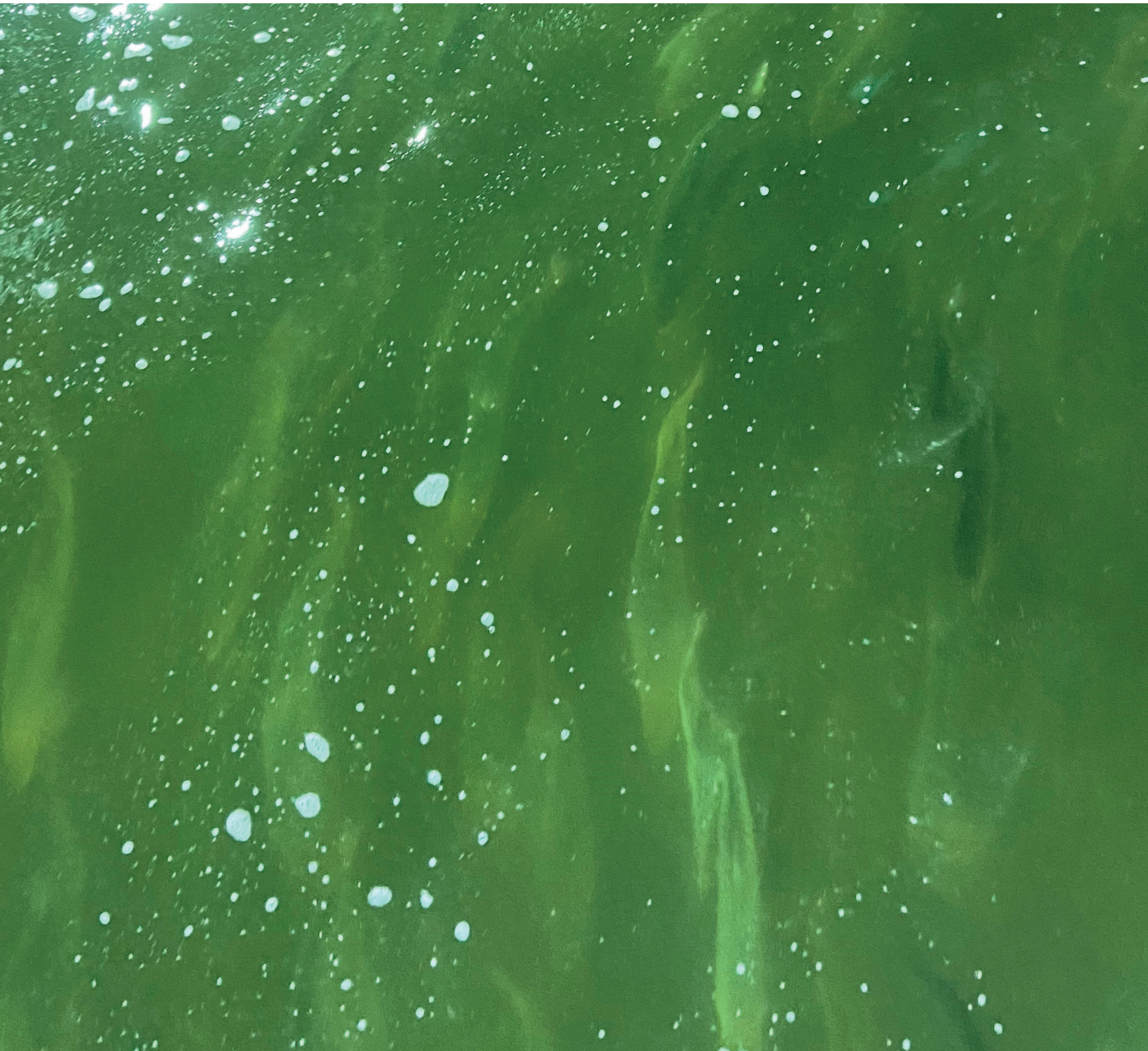

Colophon

6th NordicRAS Workshop on Recirculating Aquaculture Systems
Tromsø, Norway, 3-4 October 2023
Book of Abstracts

Edited by Johanne Dalsgaard

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The workshop is organized by the Nordic Network on Recirculating Aquaculture Systems (NordicRAS) and the Technical University of Denmark (DTU Aqua) in cooperation with University of Tromsø (UiT). In addition, session V on "Diseases and Welfare in RAS" is organized in collaboration with the Norwegian University of Life Sciences (NMBU).

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6th NordicRAS workshop on Recirculating Aquaculture Systems

Book of Abstracts

Tromsø, Norway

3-4 October 2023

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Johanne Dalsgaard	DTU Aqua, Technical University of Denmark, Denmark
Ann Wolf	DTU Aqua, Technical University of Denmark, Denmark
Per Bovbjerg Pedersen	DTU Aqua, Technical University of Denmark, Denmark
Jelena Kolarevich	UiT, The Arctic University of Norway

Session V on Diseases and Welfare in RAS was organized by David Persson, NMBU, Norway and Niccolò Vendramin, DTU Aqua, Denmark.

Correspondence:
Johanne Dalsgaard
jtd@aqua.dtu.dk

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Welcome to the 6th NordicRAS Workshop

It is a great pleasure to welcome you all to the 6th NordicRAS workshop – this time in Tromsø - the arctic capital in beautiful Norway. Hopefully, it will be some lovely and rewarding days among friends and colleges from the intriguing world of recirculation.

Coming back from a period without the opportunity to physically meet for workshops, and four years since the last NordicRAS workshop in Berlin, we were uncertain about the interest in resuming the event. No need for that - the workshop was fully booked before summer. Unfortunately, we have only been able to help a few from the long waiting list and we might therefore have to consider slightly more participants at the hopefully upcoming 7th NordicRAS workshop while keeping the intimacy and the concept of all presentations in the same room.

The great interest reflects the progressive development of the industry. While one of the initial aims of the Nordic Network on Recirculating Aquaculture Systems was to help speed up the development within RAS, we believe that the aim of facilitating the cooperation between colleges, and scientific and industrial partners, is now more relevant.

During the last years, we have witnessed a huge expansion in fish farming in RAS. Driven primarily, of course, by salmon smolt production in freshwater, but more on-growing in freshwater, brackish water and in saline water is appearing in the horizon. No doubt, RAS offers many advantages for such production as well, and if the potential risks of e.g., H2S can be addressed or eliminated, marine RAS may well undergo rapid development in the coming years.

It has been a hallmark for NordicRAS to create an opportunity for exchanging practical experiences and scientific knowledge on the newest developments in RAS. At the 6th workshop we will again have presentations covering this spectrum and we are very grateful to the many speakers from both industry and science that have agreed to give presentation here in Tromsø.

In addition, we are grateful to UiT for collaboration and assistance regarding the organization of this workshop and the technical tour, and to NMBU and the fish vets at DTU for organizing the session on fish health and welfare in RAS.

Last but not least, we are very grateful to BioMar for once again being the main commercial sponsor of the workshop and also to AG-Fisk/Nordic Council of Ministers for financial support.

We wish you all a warm welcome to the 6th NordicRAS workshop - let us together make it yet another memorable event.

On behalf of NordicRAS
Per & Johanne

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Abstracts of oral presentations

Presented at the

6th NordicRAS Workshop on

Recirculating Aquaculture Systems

Tromsø, Norway

3-4 October 2023

Challenges encountered and lessons learned operating large scale commercial RAS systems in Norway

Matthijs van den Boogaard*

SalMar Settefisk AS, Norway

Abstract

SalMar is the second biggest salmon producer in the world and is the largest farming company in Troms and Finnmark county, with activities that stretch from Harstad in southern Troms to Sør-Varanger in Finnmark. The segment has a maximum permitted biomass (MAB) of 75,116 tonnes for the production of salmon.

SalMar has 5 state of the art RAS facilities of which 2 smolt plants are located in Northern Norway. Robust and high-quality smolt is a decisive factor for the success of the entire value chain, and in 2022 the expansion of the facility at Senja was completed with the first smolt delivery in 2023. The expansion will result in increased capacity to produce more smolt, as well as flexibility to produce larger smolt.

It is possible to produce more salmon in Norway, and Northern Norway has significant potential for further growth. This region has good environmental conditions for sustainable production. The expansion of SalMar's smolt production, production capacity through NRS, as well as the new local slaughterhouse and further processing plant InnovaNor, underlines the importance of Northern Norway as a whole for the group. (https://www.salmar.no/om-salmar/vare-virksomhetsomrader/).

The presentation will focus on Salmar's experiences with producing Atlantic salmon smolt in RAS in Northern Norway.

* *Matthijs.van.den.Boogaard@salmar.no*

A brief history of RAS, from the top of Europe

Arvid Pedersen*

Grieg Seafood Finnmark, Norway

Abstract

Grieg Seafood Finnmark has since 2007 produced smolt in RAS. The last years 10 million postmolt annually, with approx 90% of this biomass in RAS tanks. The presentation will focus on Grieg Seafood's Finnmark`s experiences with producing Atlantic salmon smolt in RAS, including some thoughts on future developments.

Grieg Seafood is one of the world's leading salmon farming companies targeting 120,000 – 135,000 tonnes of harvest in 2026 produced at farms in Rogaland and Finnmark in Norway, and in British Columbia and Newfoundland in Canada (https://griegseafood.com/about-us). Grieg Seafood in Finnmark has a hatchery in Landersfjord in Lebesby, breeding facilities in Alta, Loppa, Nordkapp and Hammerfest, a butchery at Simanes in Alta, and a post-smolt facility in Hasvik. The production capacity in Finnmark is 38,000 tonnes slaughtered weight Atlantic salmon annually, divided between 28 concessions in the sea and one freshwater concession. The hatchery in Landersfjord is one of the world's most modern facilities for breeding smolt including large smolt prior to sea cages (https://griegseafood.com/finnmark).

* *arvid.pedersen@griegseafood.com*

Status, challenges and solutions related to system design and operation in RAS

Per Anthon Sæther*

Åkerblå AS, Norway

Abstract

The use of RAS technology in Norwegian salmon farming has had an explosive development in the last 10 years and has accelerated further in recent years as a result of increased investment in landbased post-smolt production. However, this increased investment has led to new challenges related to fish health and fish welfare, and an increased focus on system design and process technology in the RAS facilities as a possible reason for this.

Recycling technology has taken over as the clearly dominant production technology within land-based farming in Norway. Coinciding with this development, there has been a gradual shift in challenges related to fish health and fish welfare from infectious diseases to production disorders. Kidney calcifications and hemorrhagic smolt syndrome have been reported as the two most important causes of death in hatcheries in recent years, closely followed by a number of other production disorders such as gill cover shortening, fin damage and development of loser fish. Hydrogen sulfide poisoning is another disorder that has received much attention in the last 5 years and has led to many cases of mass death.

Based on experience from the field, it appears that many of these disorders are linked to deficiencies in system design, operational control and a general lack of understanding the salmon biology. It is of great importance as a RAS-designer to understand that the production of salmon is a biological production and that one therefore must focus on giving the fish the best possible and most appropriate environmental conditions in relation to the needs the salmon have in the various phases of its life cycle. Good water quality is a prerequisite for a stable and predictable production. The use of different input factors often has different effects on the fish's health and welfare. The focus on good water movement in tanks has been lacking for several years and the importance of water movement and the establishment of correct water inlets and outlets in tanks has contributed significantly to several of the most common production disorders in Norwegian land-based farming today. Poor water movement in fish tanks also often leads to poor self-cleaning ability, which in turn leads to the accumulation of organic material and an increased risk of poor water quality and even hydrogen sulphide poisoning. Most of these challenges can be found in all countries that practice recycling-based farming in the world today.

The solutions lie in an increased focus on smolt being produced in these facilities and not just nitrifying bacteria. The understanding that a biological production requires adaptations, both on the equipment side and through the correct use of water quality additions that support the fish's development in all parts of its life cycle, must be supported and reflected in the technical solutions and inputs that are chosen in the future.

* *peranton.saether@akerbla.no*

Innovative and effective water treatment with squeezable foam rubber filters in RAS

Bence Dániel Kovács*, Kim João de Jesus Gregersen, Lars-Flemming Pedersen

DTU Aqua, Hirtshals, Denmark

Abstract

Fixed bed biofilters are commonly used in Recirculating Aquaculture Systems (RAS) to facilitate nitrification and remove dissolved and particulate matter. The accumulation of particulate organic matter (biofilm and entrapped solids) necessitates backwashing procedures. This study included pilot scale testing of novel fixed bed biofilters, with a specific focus on their N and C removal, cleaning, and backwashing capabilities. Reticulated polyurethane foam (Levapor®) was selected as bio-carrier element, due to a number of interesting properties (i.e. material, porosity, large protected areas, and high S/V ratio).

Four foam rubber filters (16 l reactors filled with 10 l bioelements) were installed in a 20 m^3 RAS holding approx. 250 kg of rainbow trout and tested over a 4-month period. Controlled analysis of one-pass removal and mass balances of selected water quality parameters were made to determine the treatment efficiency at different hydraulic retention times (HRT). In addition, removal capacities were compared prior to and 1 hour after squeezing (backwashing) the bioelements.

From week 1 to week 14, between 55% and 90 % (71% ± 12%) TAN removal was found in filters with high HRT and between 21% and 60% (41% ± 12%) in filters with low HRT.

Additional apparent nitrite removal up to 70 % was measured in biofilters with high HRT.

Significant entrapment of bacteria was found in both filters (Bactiquant values reduced by 15%± 10% and 37%± 18% in the low and high HRT filter) and improved turbidity averaging 9%± 5% and 25%± 12% in the low and high HRT filter as well. When the biofilters were drained/squeezed, a consistent amount of dry matter was withdrawn over time (38.2 ± 1.2 g/week and 24.8 ± 0.7 g/week in low and high HRT respectively) corresponding to up to 3.8 kg/m^3 foam. The backwashing procedure slightly reduced the immediate single-pass removal capacity of the organic matter and bacteria. However, TAN and nitrite removal were unaffected or even improved following backwashing. We foresee several potential applications of the new type of biofilters and will briefly discuss pros and cons.

Acknowledgments: This research was funded by the European Maritime and Fisheries and Aquaculture Fund (EMFAF) and the Danish Ministry of Food, Agriculture and Fisheries project ”ProBiOzon” (J.no. 33111-I-21-076)

* *kovacsbenced@gmail.com*

Sound advice: Noisy information in the tank soundscapes of recirculating aquaculture systems (RAS)

Gaute A. N. Helberg[1,2*], Marianna Anichini[3], Bjørn-Steinar Sæther[1], Jelena Kolarevic[1], Chris Noble[4]

[1]UiT, Tromsø, Norway; [2]Lerøy Seafood Group ASA, Trondheim, Norway; [3]NTNU, Ålesund, Norway; [4]Nofima AS, Tromsø, Norway

Abstract

Recirculating aquaculture systems (RAS) provide the opportunity to control and potentially optimise the aquaculture production environment. There is a growing knowledge base on the effects of water quality, light, microbial composition and dynamics, and water velocity upon the health, welfare and performance of Atlantic salmon (*Salmo salar*). However, relatively little attention has been given to understanding the tank soundscapes in RAS. Pumps, water treatment equipment, feeding systems and human activity can all contribute to the soundscapes of RAS tanks. Furthermore, the salmon themselves produce sounds that can be related to feeding, to the filling of their swim bladder, or other types of behaviour.

Previous research on RAS soundscapes has mostly focused on the potentially detrimental effects of noise on fish welfare. However, the sounds could also provide information about the behaviour of the fish in the tank and the performance of the system. Feeding systems based on passive acoustic monitoring have been developed for shrimp farming, and the same principles could be adopted in RAS tanks, which would be especially useful when high turbidity impairs the possibility for vision based intelligent feeding systems.

In the current study, the soundscapes of tanks in small scale recirculating systems stocked with Atlantic salmon were investigated under a normal feeding regime and during a period of feed withdrawal. Results demonstrate temporal variations in the recorded soundscapes, that correlate with feeding events. Furthermore, drum screen filter malfunction, feeding system irregularities and issues with oxygenation were identified by variations in sound amplitude computed at specific frequency bands characterizing the tank soundscape. Combined, our outcomes highlight how an acoustic based monitoring system can provide robust and reliable information on both fish performance and system status.

Acknowledgements: This research was funded by the Research Council of Norway (RCN) and Lerøy Seafood Group ASA, as part of an Industrial PhD project (#329519). Data was collected in cooperation with the RCN project "Optimising Feed Withdrawal for Safeguarding Fish Welfare" FastWell (#295200).

* *gaute.helberg@leroy.no*

Automatic visual segmentation of fish in RAS environment using foundation models

Hilla Fred[1], Mogens Agerbo Krogh[2], Britt Bang Jensen[3], Paw Petersen[4], Laura Ruotsalainen[5], Tapio Kiuru[1], Matti Pastell[1*]

[1]Luke, Helsinki, Finland; [2]AU, Aarhus, Denmark; [3] Norwegian Veterinary Institute, Oslo, Norway; [4]OxyGuard International AS, Farum, Denmark; [5]University of Helsinki, Helsinki, Finland

Abstract

Computer vision based methods in RAS farming are under development, as the automatic tracking of the animals using cameras would allow for cost-efficient monitoring and precise collection of data on fish behavior. Changes in behavior are an indicator of change in fish health or welfare. Accurate detection of fish in images is an important building block in a system that automatically tracks the movement of the animals.

The conditions in a RAS production setting are challenging for visual monitoring, so it is very useful to develop and test the methods on real-life data. Our video data was recorded on two commercial RAS farms, one farm cultivating Rainbow trout and one focusing on Atlantic salmon, using four RGB cameras positioned over the fish tanks. For the dataset, 2654 images were extracted at different times over several days' timespan to ensure a varied dataset, and the fish individuals were manually annotated in the images using bounding boxes.

Supervised solutions for detecting fish in RAS environment have been developed that work well in a research setting, but methods that do not require training data in the target environment have not been published. This is crucial, as the amount of manual work needed to create training datasets for supervised learning limits the use of machine learning models in real-life scenarios. We aim to leverage the recent advances in instance segmentation foundation models, namely the state-of-the-art benchmark performance reaching Segment Anything model (SAM). In instance segmentation, individual objects are identified in the image, resulting in segmentation masks that cover the pixels belonging to the corresponding objects.

We study how SAM performs when prompted with hand-annotated images of fish, and how it competes against automatic mask generation. Both experiments were conducted without any task-specific training of the model. Additionally, we looked into post-processing methods for filtering out bad quality masks using properties of the mask contours. We plan to use these masks to train a faster segmentation model to work in real-time and evaluate the performance.

Acknowledgements: The research was funded by Nordic Research and Innovation Programme for Sustainable Aquaculture under the NordForsk umbrella as part of the IntelliRAS project. Annotation work was conducted by Lea Ala-Ilomäki[1], Mille Stenholt Jensen[2] and Sofie Bælum Bugge[2].

* *matti.pastell@luke.fi*

Foam fractionation in freshwater – new knowledge and future perspectives

Kim Joao de Jesus Gregersen*, Lars-Flemming Pedersen

DTU Aqua, Hirtshals, Denmark

Abstract

Foam fractionation is one of the few technologies currently available capable of removing micro particles and dissolved organic matter directly from the water in RAS. In recent times, the use of foam fractionation in indoor saltwater RAS facilities has received attention and are now implemented in most full-scale facilities. However, foam fractionation in freshwater has primarily been considered inadequate. This is mainly connected to a perceived low efficiency and high cost of operation. However, scientific studies using foam fraction in freshwater under controlled conditions are lacking.

In a replicated pilot scale trial, foam fractionation was shown to reduce particles, bacterial activity and organic matter by 50% or more. When combined with the use of ozone, foam fractionation in freshwater systems, was capable of removing 75% of organic matter and approximately 90% of particles and bacterial activity. The efficiencies obtained are similar to previous trials conducted in seawater.

In a subsequent study, a new low tech prototype foam fractionation device was developed for model trout farms. By making use of existing airlifts, installation cost and operational expenditures can be substantially reduced. Ongoing field trials utilizing the prototypes have shown a theoretical removal capacity from 25 % to almost complete removal of organic matter measured as BOD_5.

While further research is clearly needed to understand the bottlenecks of using foam fractionation in freshwater, the pilot scale results clearly show, that when operated under optimal conditions, foam fractionation in freshwater can be a powerful tool to improve water quality and the overall rearing environment. Thereby reducing the carrying capacity for microbial growth with savings on aeration and a reduction in CO_2.

Furthermore, the development of new ways to apply foam fractionation to aquaculture systems (as exemplified by the new airlift foam fractionation prototype), could lead to a large reduction in the cost of installation and operation of foam fractionation, both in fresh and seawater.

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* *jdjg@aqua.dtu.dk*

Reduced sludge nutrient discharge from freshwater Atlantic salmon (*Salmo salar*) produced in recirculating aquaculture systems

Kyla M. Zatti*

BioMar AS, Trondheim, Norway

Abstract

Biosolid waste, or sludge, is found in all RAS systems at varying levels. This by-product is nutrient dense and comprised of uneaten feed, excreta, by-products of metabolism, and bacteria. In the right proportions, sludge can be an advantageous circular resource for use in fertilizers or for biogas production. However, when levels of certain nutrients, heavy metals, or pollutants are too high the sludge becomes unfavourable and difficult to utilise as a fertilizer. With approximately 413 million salmon smolts being produced in Norway in 2021 (Fiskeridirektoratet, 2022) the estimated amount of sludge averages 12,300 tonnes dry weight, assuming an FCR of 1, with varying compositions due to differences in feed compositions (calculated based on Nofima's Report 25/2019). As a result, it is imperative to assess how raw material combinations in aquafeeds affect sludge quality and to target recipes that meet regulations for the broadest applications (Class 0 & I; Lovdata FOR-2003-07-04-951); while ensuring the highest nutrient utilization from the fish. To determine how excess nutrients and heavy metals in sludge can be reduced, a freshwater trial was conducted over 17 weeks, where 3 diets were fed to Atlantic salmon (*Salmo salar*; 19.5 ± 0.07g) in triplicates (Hirtshals, Denmark). Diets were formulated to meet commercial specifications, whereby changes to a blend of key protein raw materials were made while holding all remaining ingredients constant between treatments. Survival throughout the trial was 96%. No significant differences were found for SGR, FCR, welfare or health parameters. Regarding sludge composition, significant improvements were seen between Diet 3 compared to Diet 1, where zinc was reduced by 54%, phosphorus by 48%, iron by 29%, manganese by 36%, calcium by 8%, and nitrogen by 16%. However, significant differences were also found in Diet 2 compared to Diet 1 but not to the same extent. Furthermore, arsenic and mercury were significantly reduced in Diet 3 compared to Diet 1 by 21% and 56%, respectively, but no significant differences were found for cadmium or lead. Collectively, these results illustrate that with careful selection of raw materials during feed formulation, excess nutrients and heavy metals in sludge can be reduced while still maintaining optimal fish performance resulting in a more sustainable, lower environmental impact feed and sludge.

* *kylza@biomar.com*

Activated sludge as end of pipe treatment technology in an industrial salmon RAS, efficiencies, and production of nitrous oxide

Carlos O. Letelier-Gordo*, Maria Federica Carboni, Ching Seow

DTU Aqua, Hirtshals, Denmark

Abstract

To accomplish complete denitrification two key parameters need to be controlled: the carbon to nitrogen ratio (C:N) and the hydraulic retention time (HRT). The purposes of this study were to 1) evaluate the performance of batch denitrification reactors at different C:N ratios and define the optimal HRT for complete denitrification; 2) Evaluate through a mass balance the efficiency of N removal in an operating activated sludge End-of-Pipe (EOP), to produce 1200 tons salmon per year.

Nitrogen is mainly present in recirculating aquaculture systems (RAS) effluent in the form of nitrate (NO_3-), which is converted to nitrogen (N_2) gas through denitrification. However, heterotrophic denitrification is a four enzymatic steps process where harmful intermediates can be produced, such as nitrous oxide (N_2O), which is a greenhouse gas equivalent to 297 times CO_2. Even though farms manage to reduce nitrate and nitrite, and by this accomplishing with environmental regulations, incomplete denitrification needs to be considered and avoided to reduce the carbon footprint associated with fish production.

The batch trials showed that nitrate was reduced at different rates, and N2O was produced in the 3 reactors showing the effect of C:N. At a C:N = 6.5 the full process was completed after 8 h, while, 12 h were needed at a C:N = 4.5. The evaluation of the industrial activated sludge EOP showed that the technology treating 20% of the total discharge flow and using primarily fish organic waste as a carbon source, manages to remove 97% TN, 95% TCOD and 92% P, complying with the local environmental regulation. Attention should be paid to nitrous oxide production (2.5 kg N_2O/day or 742 kg CO_2/day) as it can affect the carbon footprint of the fish produced.

* *colg@aqua.dtu.dk*

Use of vacuum-UV radiation in fresh -and seawater RAS: Effects on water quality and development of pike perch and seabass larvae

Ivar Lund*, Kim Joao Gregersen, Manuel Gesto, Sanni Aalto

DTU Aqua, Hirtshals, Denmark

Abstract

Accumulation of micro-particles in recirculation aquaculture systems (RAS) is generated from feed waste and feces. Long retention times in RAS combined with an abundance of bioavailable organic matter and nutrients promote and proliferate microbial growth. In fish larval culture this is a typical scenario and opportunistic bacteria may become harmful and cause stress and diseases. Vacuum-UV (V-UV) is a new and powerful advanced oxidation process (AOP), which rely on UV emissions at both traditional UV-C germicidal wavelength at 254 nm as well as emission at 185 nm having a powerful oxidative capacity to form hydroxyl radicals directly with water and to be able to oxidize even dissolved slow degradable organic compounds. In two experiments in fresh - and seawater we compared the effect of V-UV radiation versus no V-UV radiation by use of 20 days post hatch (DPH) pike perch - or seabass larvae, respectively. The larvae of both species had just transited co-feeding of live feed (*Artemia*) and were during the experimental period (20-35 DPH) fed extruded larval feeds, exclusively. Larvae - and water samples were taken with a few days interval throughout the experiments. Results showed a significant effect of V-UV radiation on a range of water quality parameters (BOD, COD, turbidity, UVT, TOC and bacteria abundancy (QPCR)) both in fresh- and seawater. V-UV radiation significantly reduced the bacterial activity throughout the entire experiment in freshwater and seawater and significantly improved water transparency (UVT). Growth of pike perch larvae in freshwater was significantly and positively affected by V-UV radiation. In seawater seabass larval growth was initially negatively affected by V-UV after, but effects diminished towards end of the experiment. Microbiota activity and stress markers in pike perch – and sea bass larvae indicated an influence of V-UV. Results points to differences in the effect of V-UV in fresh – and seawater RAS.

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* *il@aqua.dtu.dk*

Particle load effect on RAS water quality and salmon welfare and performance

Paulo M. Fernandes[1*], Ludvik Fiksdal[2], Leif Bø[2], Paula Rojas-Tirado[1], Endre Steigum[1], Mark Powell[3], Irene Roalkvam[3], Melanie Andrews[3], Ingrid Bakke[4], Simen Fredriksen[4], Håkon Dahle[2], Ole-Kristian Hess-Erga [1], and Sara Calabrese [1]

[1]NIVA, Bergen, Norway, [2]University of Bergen, Bergen, Norway, [3]Marineholmen RASLab AS, Bergen, Norway, [4]NTNU, Trondheim, Norway

Abstract

Fish produced faecal material and uneaten feed add an extra organic load to recirculating aquaculture systems (RAS) that could hinder the performance of biofilters and fish. Accumulation of solids leads to extra organic material available for heterotrophic and opportunistic microorganisms to proliferate, affecting the stability of microbial communities within the RAS boundaries. It may also lead to increased oxygen consumption and carbon dioxide production and affect gill health and fish performance. There is, however, not enough evidence to conclude on all the effects that accumulated solids may have on RAS, biofilter and fish performance and welfare.

In this study, we aimed at comparing the effect of elevated solids load on baseline water quality levels, fish performance and microbiota (fish, water and biofilter) in six replicated RAS stocked with Atlantic salmon. The whole trial lasted 120 days and followed the smoltification process, covering the pre-smolt to post-smolt stages, from 1-2 ppt to 15-17 ppt. Biofilter media was changed with matured brackish water media once salinity was changed from 1-2 ppt to 15-17 ppt. The fish were subsequently transported to flow-through seawater tanks, and their performance was followed over another 96 days. Elevated solids load was created by manual daily re-addition of collected faecal material and automatic drum filter backwashing re-addition. Triplicate systems for high or low solids load were operated at 6.7 ± 0.8 mg TSS/L and 1.5 ± 0.2 mg TSS/L, respectively.

Particle load had a limited effect on baseline water quality levels, but differences between treatments were found in nitrogenous compounds (Total Ammonia Nitrogen, NO_2^-, NO_3^-), total and dissolved organic carbon, microbial activity (k-value) in the biofilter elements, and particle size distribution (PSD) metrics. There was a slight tendency for increased feed intake, specific growth rate and a higher cumulative feed burden in the low load systems. By the end of the RAS phase, this led to heavier fish in the low load group, compared to the high load group. Analysis of smoltification markers and general stress indicators is ongoing and will be discussed during the presentation. The microbiota results will be presented separately.

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[*] *paulo.fernandes@niva.no*

Nitrification at high salinities: nitrification capacity, salinity tolerance, and key players in the nitrifying communities

Line Moen, Fernando Fernando, Ingrid Bakke*

NTNU, Trondheim, Norway

Abstract

The interest in fish production in high salinity RAS is increasing in Norway. The first RAS for production of Atlantic cod was recently implemented, and there are now several commercial RAS for production of post-smolts of Atlantic salmon. The start-up of nitrifying biofilters in marine RAS is often considered to be a challenge. In this study, we aimed to improve the understanding of the nitrifying communities in RAS biofilters operated at high salinities.

We investigated the maximum nitrification capacity and the tolerance to salinity fluctuations for biofilm carriers originating from two RAS; RAS1 for production of posts-molts, operated at 15 ‰ salinity, and RAS2 for production of Atlantic cod, operated at 35 ‰ salinity. RAS2 had previously been used for production of post-smolt (22 ‰ salinity), and the biofilter was originally started by using carriers from the RAS2 as inoculum. We further characterised the bacterial and archaeal communities of the carriers and examined the temporal development of the nitrifying communities following a transition to full seawater salinity operation.

The maximum nitrification capacity of carriers originating from RAS1 was three times higher than that for those originating from RAS2 and was found to be 0.44 mg N L^{-1} min^{-1} m^{2-1} at 15 ‰ salinity. Surprisingly, the high nitrification capacity was maintained in both freshwater and seawater. Furthermore, we observed an apparent loss of nitrogen from the batch reactors during the experiments. Characterisation of the bacterial communities revealed that nitrifiers accounted for around 15% of the total reads for the most active biofilm carriers. The nitrifying communities were dominated by two ASVs related to *Nitrospira salsa*, but the proportion of bacterial ammonia oxidisers was low. We further characterised the archaeal communities and found that they were almost completely dominated by one ASV classified as *Nitrosopumilus*. Members of this genus are known to be ammonium oxidizers with a strong affinity for ammonium at low concentrations. Next, we amplified and sequenced the *Nitrosopumilus amoA* gene, which was found to be highly similar to the *amoA* gene sequence previously reported for *N*. *oxyclinae*. *Nitrosopumilus maritimus* has recently been shown to convert ammonium via nitrite to dinitrogen gas accompanied by intracellular O_2 production. Further studies are required to determine whether the observed nitrogen loss from the batch reactors was due to technical problems associated with measurements of nitrogen compounds, or whether it can be explained by the N_2 production by *Nitrosopumilus* in the biofilm communities.

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* *ingrid.bakke@ntnu.no*

High resolution real-time monitoring of dissolving gasses in RAS water: A case study of dissolved gasses dynamics from smolt production

Raju Podduturi*

AKVA Group Land Based A/S, Frederica, Denmark

Abstract

The partial pressure of dissolved gasses and gasses composition in rearing water has significant impact on fish performance and welfare in RAS. In RAS, there is continuous oxygen consumption and CO_2 release due to respiration and metabolism of fish and microorganisms. Excessive CO_2 levels, limitation of oxygen levels and super saturation of nitrogen gas in rearing water are lethal to fish. Along with these gasses, a continuous occurrence of acutely toxic H_2S gas as result of microbial processes that could potentially cause mass mortalities of fish in RAS systems has also been reported in the industry. Despite the critical importance of dissolved gasses on fish welfare and mortality, knowledge on the dynamics of dissolved gasses, their composition and mass balance in RAS system is limited.

The present study investigates the composition of dissolved gasses (CO_2, O_2, N_2 and H_2S), total gas pressure (TGP) in RAS water and physiochemical water quality across the water treatment module in a smolt production system using a high throughput real-time gas and water quality monitoring sensor system (AquaSense, SeaRAS). The study period covers a smolt production cycle of 12 weeks with varied feeding and biomass levels. The water treatment module consists of mechanical filtration, fixed bed biofilters, ozonation, diffused aeration basins for CO_2 removal and oxygen cones.

The obtained results will provide insights into dynamics of dissolved gasses in fish production and in between each water treatment process and the impact of physiochemical water quality on the dissolved gasses dynamics. From the results obtained, a gas-mass balance model including gas production, consumption and removal in each water treatment compartment will be developed. The study's objective also includes the assessment and optimization of CO2 removal and oxygenation efficiencies for varied system load in feed and biomass, thus optimizing energy consumption in RAS operations.

* *rpodduturi@akvagroup.com*

Effects of chronic and fluctuating CO_2 levels on the production performance of rainbow trout

Peter Vilhelm Skov[1*], Helene Volkoff[2], Tilo Pfalzgraff[1]

[1]DTU Aqua, Hirtshals, Denmark; [2] Department of Biology, Memorial University, St. John’s, Canada

Abstract

The release of metabolically produced carbon dioxide (CO_2) in RAS is inherently linked with the oxygen consumption of fish and bacteria, and accordingly fluctuates strongly on a daily basis. Most RAS facilities are equipped with degassing installations, but during peak rates of CO_2 excretion, they may not be able to maintain normocapnic conditions. Consequently, dissolved CO_2 levels build up during the day when the metabolic rate of fish and biofilters are at a maximum and return to baseline conditions during the night.

The majority of studies that have examined the effects of CO_2 on fish have applied experimental conditions under which fish are subjected to constant elevated levels of dissolved CO_2. Based on measurements from different RAS facilities, we show that CO_2 levels may fluctuate by as much as 20 mg/L over the course of a day. We therefore examined the effects of CO_2 levels on appetite, growth and feed utilization under 3 experimental regimes: constant levels of 10 and 25 mg CO_2/L, and fluctuating between 10 and 25 mg CO_2/L on a daily basis, against a normocapnic control group. Experiments were conducted in both freshwater and seawater, using diets containing different levels of phosphorous to assess implications for the occurrence of nephrocalcinosis.

CO_2 had a significantly stronger effect on growth and feed utilization in seawater than in freshwater. Fish willingly accepted their daily rations (1.3%), but growth rate and feed conversion were significantly affected in both. Daily fluctuations in CO_2 appeared to be less severe than chronic exposure, possibly because high CO_2 levels did not occur until late in the afternoon when feeding was completed. All CO_2 treatments resulted in reductions in maximum voluntary feed intake, which was further supported by changes in gene expression levels of appetite regulating hormones. CO_2 levels and dietary phosphorous did not lead to clear pathological signs of nephrocalcinosis.

* *pvsk@aqua.dtu.dk*

Why RAS will be a greater part of future fish farming

Jens Petri Petersen*

Skagen Salmon, Denmark

Abstract

Skagen Salmon is a land-based salmon RAS facility designed to produce 3,600 ton market-size salmon/yr from egg to 4 kg harvest size. The facility is situated just outside the city of Skagen, on the northern tip of Denmark, with access to clean seawater from the North Sea. Intake water (make-up water) and recirculating water is treated by mechanical filtration, Ozone, and UV. Reject water is treated on site through flocculation and denitrification, and sludge is used for biogas in a next-door biogas facility operated by the public sewage plant. Part of the electricity consumption is provided by solar panels and an existing wind turbine on the site.

Eggs are received four times a year. Fish are smoltified at 100 g and grown to 4 kg in 6 alike systems, the whole cycle taking just about 2 years. (https://www.skagensalmon.com/).

The presentation will explain the reasons why we believe the farming of fish in RAS plants will grow and will represent a greater and greater part of future fish farming.

* *jpp@skagensalmon.com*

Growth in land-based aquaculture of Atlantic salmon in Iceland

Helgi Thor Thorarensen*

UiT, Tromsø, Norway

Abstract

The history of land-based aquaculture of salmonids in Iceland goes back over thirty years. Current production capacity of operating land-based fish farm is about 10 600 mt, while the total production in 2022 was about 6 800 mt, mainly Arctic charr (4 900 mt) and salmon (1 900 mt). Some of the discrepancy between production capacity and licences is explained by recently completed expansions existing farms where biomass is developing. These fish farms are using hybrid systems where water is oxygenated, CO_2 is degassed and water may be reused. However, none of the farms use recirculation aquaculture systems (RAS) with biofilters and there does not appear to be any interest in those for on-growing among Icelandic producers. There are three companies that are responsible for most of the production. However, Samherji is by far the largest (80% of the production) operating three land-based farms and with a new farm under construction.

There is a growing interest in land-based aquaculture of salmon and salmonids in Iceland as evident from plans for total expansion of annual production by as much as 120 000 - 130 000 mt, although the plans for initial phases of development are only about half of that. In comparison, the total production of salmon in net-pens in Iceland was 43 000 mt in 2022. Most of the new fish farms are located in the Reykjanes peninsula, close to Keflavik airport with connections to North America, and the harbour in Þorlákshöfn where fish can be shipped in containers to Europe within 72 hours.

Salmon will be the primary species produced on the new farms, while some aim also for rainbow trout or Arctic charr, however, the farms will suit all three species. Five different companies are building or planning to build as many fish farms. Some of these projects have started construction, while others are still securing finance and completing environmental assessments. The new projects clearly build on the bank of experience in land-based production in Iceland. None will use RAS systems for on-growing, although one of the farms will use a RAS system for smolt production. Most of the farms will use bore hole seawater that has filtered through the lava layers in Reykjanes and reached a temperature of about 7 °C. One farm will use the cooling water from a geothermal power plant. The production methods have been used for over thirty years in land-based fish farms in the region and have proven successful and returning profit.

* *helgi.t.thorarensen@uit.no*

Atlantic salmon RAS in the Faroe Islands

Lisbeth Lindenskov Joensen*

Bakkafrost, Faroe Islands

Abstract

Bakkafrost was established in 1968 and has grown to be the largest salmon farming company in the Faroe Islands and the third-largest fish farming company in the world. Bakkafrost has its own fish feed company (Havsbrún), producing feed including fish meal and oil from locally caught sustainable pelagic stocks along with Non-GM plant-derived proteins and lipids. Short distances from fishing grounds to the feed production plant using super-cooling well-boats ensure high ingredient freshness, complete traceability, and a low CO_2 footprint.

Bakkafrost has several land-based RAS based hatcheries spread throughout the Faroe Islands reducing risks and ensuring stable delivery of smolt. Bio-waste from the hatcheries is used for biogas production currently providing 1900 homes in the Faroe Islands with electricity, and leftovers from the biogas production are used as fertilizer. Bakkafrost is continuously increasing its hatchery capacity aiming at increasing the size of smolt on land and reducing the time and associated biological risks at sea. (https://www.bakkafrost.com/en/).

The presentation will focus on Bakkafrost's experiences with producing Atlantic salmon smolt in the Faroe Islands.

* *lijo@Bakkafrost.com*

Finnforel, ultimate circularity

Tommi Makinen*

Finnforel, Finland

Abstract

Finnforel produces rainbow trout in fully closed RAS from egg to harvest size using water from Lake Saimaa. The intake water is filtered, disinfected and oxidized, removing all solids > 0.02 mm. Within the facility, water is recycled through a purification system twice an hour, including mechanical filtration and biofiltration. The water is continuously disinfected, ozonated, oxidized and temperature adjusted before returned to the tanks. Discharge water is treated by Stora Enso's treatment plant. All fish are grown, processed, filleted and packed on site and transferred to retail stores on the day of slaughter. All parts of the fish are used. Cutting pieces resulting from filleting are used in fish patties, bones for broths and sauces, while residues are used in pet food. (https://www.finnforel.com/).

The presentation will focus on Finforel's experiences with producing rainbow trout in RAS including views on ultimate circularity and challenges in the End-to-End supply chain from feed to breeding and grow-out and processing.

* *tommi.makinen@finnforel.com*

Swedish RAS sturgeon farming – competing with global open-water sturgeon farms against all odds

Torbjörn Ranta*

Arctic Roe, Sweden

Abstract

Arctic Roe is a Scandinavian sturgeon RAS farm located in Småland, Sweden, producing Black caviar via the live-method. The company was founded in 2015 and is located in an abandoned paper mill along one of Sweden's major rivers. Fish farming is highly regulated in Sweden and although sturgeon used to live in the Baltic Sea and in Scandinavian rivers, it is considered an invasive species. It is therefore so far prohibited to farm sturgeon in open water. The company uses water from its own wells while heat comes from the central heating grid in the town of Strömsnäsbruk. In addition, the company has its own cooling system and solar panels on the roof. Arctic Roe currently breeds only Siberian sturgeon (*Acipenser baerii*) but is allowed to breed five different sturgeon species. (www.arcticroe.com). The presentation will focus on Arctic Roe's experiences of producing Black caviar from sturgeon in RAS incl. challenges and future perspectives.

* *torbjorn.ranta@tranta.se*

15 years of RAS experiences with pikeperch in DK and CH

Martin Vestergaard*

SWIFISH AG, Switzerland

Abstract

SWIFISH AG was founded in 2015 in Lyss in Switzerland with the aim of producing pikeperch fingerlings. In 2020, SWIFISH expanded its portfolio with a RAS farm in Valais for producing edible fish for wholesale and retail. The RAS facility was originally designed for producing caviar from sturgeon, has a pool capacity of 1800 m^3 distributed over nine individual circuits. The facility has been converted into a modern RAS pikeperch farming facility.

The presentation will describe the production cycle, different production systems, challenges and achievements in the past 15 years. Pros and cons of farming pikeperch in RAS will be discussed.

* *vestergaard@swifish.ch*

Growing yellowtail kingfish in saltwater RAS – the story so far

Bram Rohaan*

Kingfish Zeeland, Netherlands

Abstract

Based in the Dutch province of Zeeland, Kingfish Zeeland produces yellowtail kingfish in RAS, available fresh from the Netherlands all year round with deliveries across EU. The facility includes a hatchery section, growing yellowtail from eggs to juveniles, and a farming section raising fish to consumption size. The facility uses make-up water from the Eastern Scheldt estuary (flowing into the North Sea), and energy is obtained from 100% renewable sources including wind-power, solar panels, and biogas. Established in 2015, Kingfish Zeeland has vast experience growing yellowtail kingfish in RAS. (https://www.kingfish-zeeland.com/). The presentation will describe the system and the production cycle, and the story so far of farming yellowtail kingfish in RAS on land will be shared.

* *b.rohaan@kingfish-zeeland.com*

Fish health and welfare in RAS – Experiences from production in Lerøy's RAS facilities

Julia Fossberg Buhaug*

Lerøy Seafood Group, Norway

Abstract

Lerøy Seafood Group is a world-leading seafood corporation with a history going back to 1899. The Group´s core business is the production of salmon and trout, catches of whitefish, processing, product development, marketing, and sale and distribution of seafood.

Lerøy is involved in every stage of the production of salmon and trout. We have an integrated value chain from production of broodstock and roe, to the farming of smolts and slaughter-sized fish, and all the way to the distribution of our products to customers in more than 80 countries worldwide.

The salmon is produced in three different regions along the Norwegian coast, with about 85 % of the production of smolts and postsmolts coming from farms with RAS-technology. Lerøy has invested heavily in RAS-technology in the last decade, and this has led to a much more stable and reliable smolt delivery to our sea farms. Fish health and welfare are of utmost importance in all our production, and there is more and more scientific evidence of the importance of early life stages and how they impact welfare and growth at sea. There are still many challenges in land-based production, and some of these will be addressed in the presentation.

* *Julia.buhaug@leroy.no*

Biofilter disinfection increases the colonization success of opportunistic bacteria in the recirculating aquaculture system (RAS)

Fernando Fernando*, Kristine Hofstad, Sujan Khadka, Olav Vadstein, Ingrid Bakke

NTNU, Trondheim, Norway

Abstract

There is concern that the biofilm in the nitrifying biofilters can act as a reservoir for pathogenic bacteria in recirculating aquaculture systems (RAS); thus, disinfection of the biofilter after each production cycle is currently considered a method to prevent disease outbreaks. Whether regularly disinfecting the biofilters is a proper bacterial management practice in the RAS is still controversial and is an ongoing debate among RAS operators, scholars, and the authority.

This study investigated the effect of biofilter disinfection on the colonization success of opportunistic bacteria in RAS. We ran a fish experiment in two identical small laboratory scale RAS (mini-RAS), one running with disinfected biofilter and the other with a non-disinfected biofilter (control, i.e., a mature nitrifying biofilter). Afterwards, both systems were challenged by introducing four bacterial strains, previously isolated from salmon fry, representing the genera *Proteus*, *Pseudomonas*, *Psychrobacter*, and *Flavobacterium*. Each bacterial strain was added to both mini-RAS at a final concentration of about 1.25×10^5 cells/mL. The bacterial communities of the rearing water and biofilters' biofilm were characterized by Illumina sequencing of 16S rDNA amplicons, and the relative abundances of the ASVs representing the four added bacterial strains were determined.

Our results revealed that the relative abundance of the added bacterial strains was significantly higher in the biofilters' biofilms and rearing water of mini-RAS with a disinfected biofilter, indicating the higher colonization success of the strains in that system. On day 8 post-challenge, in the biofilm samples of the disinfected biofilter, the average relative abundances of *Flavobacterium*, *Pseudomonas*, and *Psychrobacter* isolates were 4.0 ± 1.3%, 1.79 ± 0.11%, and 0.23 ± 0.05%, respectively, while the control (mature) biofilter showed relative abundances not exceeding 0.006%. The bacterial communities in the biofilm carriers and rearing water in the mini-RAS with a disinfected biofilter also exhibited lower stability over time. Furthermore, in terms of proportions, we observed a reduction of nitrifiers and an increase of Gamma-proteobacteria in the bacterial communities of the biofilm and the rearing water of mini-RAS with a disinfected biofilter.

Our findings suggested that biofilters disinfection may increase the risk of opportunistic bacterial invasion in RAS. The results can contribute to formulating better biofilter management practices to optimize the bacterial communities in the RAS and ultimately promote the fish's health and welfare.

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* *fernando.fernando@ntnu.no*

Effects of common disinfection treatments on microbial diversity and composition of fluidized sand biofilters

Christine Lepine[1*], Julianna Stangroom[2], Jordan Poley[2], Jason Stannard[3], Melissa Allen[3], Robert Baldwin[2], Adriana Artiles[3], Travis May[1], Kayla Fairfield[1], Anna Knight[1], Carlo Lazado[4], Lill-Heidi Johansen[5], Åsa Maria Espmark[6], Brian Vinci[1], and Christopher Good[1]

[1]The Conservation Fund Freshwater Institute, Shepherdstown, United States; [2]The Center for Aquaculture Technologies, Victoria and Souris, Canada; [3]The Center for Aquaculture Technologies, San Diego, United States; [4]Nofima, Ås, Norway; [5]Nofima, Tromsø, Norway; [6]Nofima, Sunndalsøra, Norway

Abstract

Disinfection is an essential element of an aquaculture facility's biosecurity, playing a critical role in pathogen reduction within recirculating aquaculture systems (RAS). While its significance is generally agreed upon among farmers, and several recommended standard operating procedures (SOP) exist, many commonly used protocols have never been experimentally validated.

Using currently accepted SOPs for RAS disinfection treatment as a guide, this study compared the microbiomes impact of three disinfection chemicals - calcium hypochlorite ($Ca(ClO)_2$), hydrogen peroxide (H_2O_2), and peracetic acid (PAA) - within pilot-scale fluidized sand biofilters. Each biofilter (n = 12 biofilters; 4 biofilters per treatment) received ammonia-rich water from a nearby semi-commercial scale RAS and was coupled via PVC piping to recirculation sumps (63 L total system volume), simulating culture systems. During the disinfection process, both $Ca(ClO)_2$ and PAA treatments met threshold concentration recommendations, though the recommended $Ca(ClO)_2$ concentration was not maintained for the required timespan. Conversely, H_2O_2 disinfection failed as the operator could not achieve recommended concentrations for disinfection using the current SOP.

Total genomic DNA extraction and bacterial 16S ribosomal RNA analysis of pre- and post-disinfection biofilm, sampled from (i) biofilter effluent piping, and (ii) within the sand beds, identified microbial communities and assessed disinfection efficacy. Both $Ca(ClO)_2$ and PAA treatments resulted in decreases in absolute abundance and shifts in relative abundance, indicative of effective disinfection. Distinct clustering on principle coordinate analysis graphs also demonstrated that post-disinfection microbiome populations in efficacious treatments differed. Despite confirmed disinfection efficacy, alpha diversity did not change during any chemical disinfection, indicating that no treatment was fully lethal by the disinfection protocols used in this study.

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[*] *clepine@conservationfund.org*

Bioaerosols as a potential transmitter of fish pathogens in an Atlantic salmon Recirculated Aquaculture System (RAS) smolt farm

Dhiraj Krishna[1,2*], Petra Elisabeth Petersen[1], Maria Marjunardóttir Dahl[1], Nicolina Vest[1], Debes Hammershaimb Christiansen[1]

[1] National Reference Laboratory for Fish and Animal Diseases, Faroese Food and Veterinary Authority, Faroe Islands; [2] Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark.

Abstract

Pathogen transmission in aquaculture is mainly through two modes, vertically via eggs and horizontally via water. Aerosols represent another potential mode of transmission. Although it is indisputable that human and animal pathogens are disseminated frequently via aerosols, there is little knowledge of aerosol-mediated transmission in aquaculture.

The Faroe Islands are known for their high-quality Atlantic Salmon produced in closed containment systems (CCS) called Recirculated Aquaculture Systems (RAS), wherein the water gets constantly cycled through various compartments. The system also involves the constant import of large volumes of air for degassing purposes, and recent studies have shown that indoor systems generate pathogen-laden aerosols from water. The sea surface microlayer (SML) has been identified as a source of aerosols that could transmit bacteria and viruses that can stay infective and transmit through long distances. In our study, we will focus on the aerosolization potential of five fish pathogens in a commercial Atlantic Salmon RAS smolt farm: Salmon Gill Pox Virus (SGPV), Infectious Salmon Anaemia Virus (ISAV), Infectious Pancreatic Necrosis Virus (IPNV), Piscine Orthoreo Virus (PRV), and Flavobacterium psychrophilum**.** The study utilized two cyclonic aerosol samplers: the Coriolis Micro wet cyclonic and Compact dry cyclonic samplers (Bertin Instruments, France). Additionally, both the fish and water were sampled simultaneously.

Preliminary findings have confirmed the presence of SGPV, ISAV, IPNV, PRV, and F. psychrophilum in the air at various Ct-values between 26-36. The RT-qPCR results have highlighted an intriguing observation of different pathogen levels in the air that fluctuated throughout the sampling period. The pathogen levels in the water directly impacted the levels in the air. However, the levels varied during the infection cycle, with lower Ct values during the peak infection in fish and the corresponding viral load in the rearing water. Various other factors, such as the sampling location, proximity to air vents, and machinery, influenced the aerosolization of the circulating water. Thus, it is imperative to maintain a high level of biosecurity in closed containment systems to prevent the uncontrolled transmission of pathogens between various RAS systems within a smolt farm.

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[*] *dkr@hfs.fo*

Fish health and welfare in RAS - challenges and opportunities

Morten Lund*, Erik Sterud, Frode Finne-Fridell

Pure Salmon Technology, Sandefjord, Norway

Abstract

Pure Salmon Technology (PSTech) is one of the largest RAS-suppliers in Norway, having over 15 years of experience with designing and constructing RAS-facilities for salmonid aquaculture. Over the past 5 years there has been an increasing focus on fish health and welfare when designing and constructing equipment and rearing facilities for salmonids. In PSTech's Fish Welfare department, we put the fish in the front seat during the design and construction phases of RAS. Furthermore, by aiming for an optimal hygienic design of the RAS-facilities, Pure Salmon Technology have significantly improved the success rate of a washing and disinfection of a RAS.

The annual report of 2022 from the Norwegian Veterinary Institute (Sommerset et al, 2023) shows that the most important causes of reduced fish health and welfare in Norwegian hatcheries/smolt facilities are production related disorders. However, the report doesn't discriminate between flow through and RAS facilities. The Fish welfare department in Pure Salmon Technology therefore performed a similar survey in 2023 on RAS-facilities to better understand the fish health and welfare status in RAS.

The PSTechfish health and welfare survey showed large variations in causes of reduced health and welfare between sites, years and even batches of fish and departments within the same site. Production related disorders had in general moderate impact on fish health and welfare, and infectious diseases also had a variable impact varying between sites. The variation between sites may reflect variation in design, but also variations in operational routines and acute changes in the water quality within the site or in the water source. Improved operational support of a RAS-facility by water quality monitoring and process-control will give the operators a tool to reduce the risk of operational causes of reduced fish health and welfare. Furthermore, our impression is that the physiological and developmental causes of reduced fish health and welfare is primarily explained by the ever intensification of the production in the hatcheries.

We may have reached the physiological limits of the salmon. Reduced temperature during production is one measure that is used to reduce the production intensity. I mean that we need to discuss other measures as well if we are to reduce the production intensity and improve the health and welfare of the fish. Sustainability in salmon production depends on this.

* *morten.lund@puresalmontech.com*

Dissecting complex disease case in RAS by a validated microfluidic chip targeting salmonid pathogens

Juliane Sørensen[1], Giulia Zarantonello[1], Alejandra Villamil Alonso[1], Simon Brøndgaard Madsen[2], Argelia Cuenca[1], Lone Madsen[1], Tine Moesgaard Iburg[1], Niccolò Vendramin[1*]

[1]DTU Aqua, Lyngby, Denmark; [2]Veterinarian, Højslev, Denmark.

Abstract

The production of rainbow trout (*Oncorhynchus mykiss*) in RAS system is intense. It normally involves high biomass in contained farm environments, high reutilization of water, and frequent introduction of new fish batches. This diverge from the paradigm of "all-in all-out" which has been a cornerstone of biosecurity and brings a number of health challenges for farmed stocks.

Along with known production bacterial and viral pathogens, new ones colonize this ecological niche. It is the case of *Piscine orthoreovirus* genotype 3 (PRV-3) was first reported in Denmark in 2017 in association with disease outbreaks with high mortality in rainbow trout. A surveillance conducted in 2017-2019, showed that although this was widespread in farmed rainbow trout, disease outbreaks with mortality were only observed in recirculating aquaculture systems, and primarily during the winter.

Importantly, preliminary field investigations have highlighted the presence of other pathogens during PRV-3 associated disease outbreaks. In order to deepen the understanding of PRV-3 associated disease, we developed and validated a high-throughput qPCR method (Fluidigm) for simultaneous detection of multiple pathogens. From March to September 2022, monthly sampling was performed from 10 fish from the same cohort. Along with tissue samples of heart and spleen, gill tissues and water were sampled. Fish were examined with traditional diagnostic methods, and results compared to the output of the microfluidic chip. Additionally, production data was recorded during the seven months, including weight, feeding, disease outbreaks, treatments, and water quality parameters. A severe disease outbreak affected the cohort we followed, the event lasted 5 weeks with an overall mortality yielding more than 2000 kg. Besides the constant presence of PRV-3, and the sporadic detection of *Yersinia ruckerii* and *Flavobacterium psychrophilum*, assays targeting Piscichlamydia and Brachiomonas yielded positive results in gill tissues, in connection with the outbreak.

A comprehensive diagnostic overview combined with the production data will be presented. The study show how disease outbreaks in RAS are complex and sustained by co-infections and possibly stressors induced by farm practices. Interestingly, mortality increased significantly after fish transfer and was not mitigated by water treatment with salt. The tool we have developed and validated can provide important information on solving complex disease cases and suggest adequate preventative measures and mitigating strategies.

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* *niven@aqua.dtu.dk*

Reducing fish weight loss using capacitive deionization during purging

Rasmus J. Jensen[1*], Jeppe Lund Nielsen[2], Karthik Laxman[3], Joydeep Dutta[4], Mark Bayley[1]

[1]AU, Aarhus, Denmark; [2]AAU, Aalborg, Denmark; [3]SWT, Stockholm, Sweden; [4]KTH, Stockholm, Sweden

Abstract

Currently, intensive RAS production involves the risk of off-flavor accumulation requiring purging of fish in clean flow through systems for up to 14 days with feed withdrawn. While this effectively removes the off-flavor, the harvest ready fish also suffer significant weight loss during this period with associated value loss.

Capacitive deionization (CDI) technology has been developed for water deionization and has now reached an efficiency where it is routinely used in a variety of water treatment scenarios including sterilization and drinking water production from brackish water. This is achieved with very low power consumption. Here we test the feasibility of using the CDI for ammonia removal to allow for continued feeding during purging to avoid weight loss. We also evaluate the removal of off-flavor molecules to improve the purging process.

We have demonstrated by running the CDI at different ammonia loads that it can control ammonia levels simulating fish densities commonly found in commercial RAS. Similarly, we have used the CDI to simulate purging regimes with maintenance feeding levels using rainbow trout (*Oncorhynchus mykiss*). These data will be presented.

As the technology is completely scalable it can be implemented into specialized high-flow RAS units intended for off-flavor removal. In this system maintenance feeding can continue to avoid weight loss while the up-scaled CDI eliminates ammonia build up. The CDI concentrates ammonia in a small volume of water that may subsequently be used as plant fertilizer. Since bacteria typically carry surface charge, they will likewise be removed from the CDI treated water.

Results from our small-scale RAS system offer a promising proof of concept for improving purging in commercial RAS.

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* *rasmus.juul@bio.au.dk*

Part-time advanced oxidation process and hydrogen peroxide treatments in recirculating aquaculture systems to reduce off-flavor compounds

Samu Pettersson[1*], Petra Lindholm-Lehto[2], Jani T. Pulkkinen[2], Jouni Vielma[2]

[1]University of Jyväskylä, Jyväskylä, Finland; [2]Luke, Helsinki, Finland

Abstract

Aquaculture is becoming increasingly important for the world's food production, the means to make it more sustainable and environmentally friendly are in great demand. Recirculating aquaculture system (RAS) offers reduced water requirement and better possibilities for waste handling. Unfortunately, off-flavors can be formed in RAS which concentrate in fish flesh. These compounds cause earthy, musty or other unwanted flavor to fish flesh which consumers find objectionable. Typically, off-flavors are removed by depurating the fish in clean water, but it often takes form days to weeks to fully remove these unwanted flavors and causes additional costs to fish producers. Therefore, reliable methods to reduce the need for depuration are desperately needed.

In this study, very intensive part-time treatments of advanced oxidation process (AOP) with a combination of ozone and hydrogen peroxide ($O_3 + H_2O_2$) and hydrogen peroxide were applied in nine identical experimental RASs rearing rainbow trout (*Oncorhynchus mykiss*). Treatments (AOP, H_2O_2, control, 3 replicas for each treatment) were applied for eight hours per day and continued for two weeks. To identify the effects, water samples were taken daily before and after the daily treatment period. Additionally, fish samples were collected before and after the experiment. In total, 14 selected off-flavors compounds were analyzed with a validated method based on SPME and GC-MS/MS. We hypothesized that the intensive addition of oxidants would lead to formation of reactive agents and degradation of off-flavor compounds.

The results showed that the treatments significantly reduced almost all of the studied off-flavor compounds in water and in fish. The AOP treatment was more efficient compared to H_2O_2 treatment, although with only a minor difference. 2-methyl isoborneol (MIB) was reduced by 65.0 ± 1.2 % and geosmin (GSM) by 37.9 ± 19.0 %. However, the overall changes in fish flesh were smaller. MIB was reduced by 20.8 ± 5.8 % in fish and GSM by 26.4 ± 17.1 %. The results showed that intensive oxidant treatments reduced the off-flavor concentrations in circulating water and in fish which can potentially lead to reduced depuration time and production costs. In the future, optimization of the treatment may have potential to even better off-flavor removal efficiencies.

[*] *samu.j.pettersson@jyu.fi*

Consequences of prolonged exposure to hydrogen sulphide on the health and welfare of Atlantic salmon (*Salmo salar* L.)

Carlo C. Lazado[1*], Bosco Ara Diaz[1,2], Ilona Nicolaysen[1,3], Mohammad Salman Malik[1], Hanna Ross Alipio[1,2], Britt Kristin M. Reiten[1], Julie Bergstedt Hansen[4], Kevin T. Stiller[1]

[1]Nofima, Tromsø, Norway; [2]Wageningen University & Research, Wageningen, The Netherlands; [3]University of Bergen, Bergen, Norway; [4]DTU Aqua, Hirtshals, Denmark

Abstract

The study evaluated the effects of prolonged exposure to previously known sub-lethal levels of hydrogen sulphide (H_2S), *i.e.*, 1 and 5 µg/L in Atlantic salmon. In Trial 1, full-strength seawater-adapted salmon were exposed continuously to H_2S for 12 days and physiological responses were determined at termination. In Trial 2, brackish water-adapted salmon were exposed continuously to H_2S for 4 weeks. Thereafter, fish were allowed to recover for 2 weeks before they were subjected to a handling-confinement stress test. Physiological responses were determined at weeks 2 and 4 of the exposure period and after a 2-week recovery. For both trials, unexposed fish served as control.

In Trial 1, no mortality was recorded following exposure. Gene expression profiling showed that mucosal organs (i.e., gills, skin and olfactory rosette) exhibited differential sensitivity to H_2S, with gills and olfactory rosette being highly responsive. H_2S significantly affected the expression of genes relevant to stress response, apoptosis/xenobiotic metabolism, and immunity, but a dose-dependent profile could not be conclusively established. The mucosal structures of the three target organs were not significantly altered by H_2S exposure. Spatial localisation of key sulphide detoxification genes (i.e., *sqor1*, *sqor2* and *suox*) revealed that mucosal surfaces have the molecular repertoire to detoxify H_2S. In addition, proteomes of skin and gill mucus were altered by H_2S exposure, where many of the proteins affected were involved in immunity and metabolism.

At the termination of trial 2, cumulative mortality in 1 and 5 µg/L groups were recorded at 4.7 and 16 %, respectively. Increased opercular ventilation and erratic swimming patterns were observed in the 5 µg/L group. The weight of H_2S-exposed fish was at least 20% lower than the control group at week 4. Transcriptome-wide profiling revealed alterations following H_2S exposure in the three mucosal organs. Genes involved in immunity, mucosal integrity and metabolism were mostly affected, and the magnitude of change was highest in the olfactory rosette. Vacuolisation and an increased number of mucus cells in the olfactory lamella were observed in H_2S-exposed fish. The gills showed minimal structural alterations, and recovery was observed two weeks after terminating the H_2S dosing. Metabolomics showed that H_2S exposure resulted in systemic but not mucosal dysregulation. The handling-confinement stress test following recovery revealed that chronic exposure to 5 µg/L significantly reduced the ability of salmon to mount an appropriate cortisol response.

The studies elucidated the physiological consequences of prolonged exposure to H_2S, thus, providing insights into how Atlantic salmon interact and respond to this enigmatic toxic gas.

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* *carlo.lazado@nofima.no*

Contingency measures for the occurrence of hydrogen sulfide: potential for hydrogen peroxide as an oxidizing agent

Julie Hansen Bergstedt*, Peter Vilhelm Skov, Carlos Octavio Letelier-Gordo

DTU Aqua, Hirtshals, Denmark

Abstract

The increasing demand for sustainable aquaculture practices has led to the widespread adoption of recirculating aquaculture systems (RAS). While RAS offers several benefits, including reduced water usage and improved biosecurity, the production of fish in RAS is not without challenges. One specific concern is the accumulation of hydrogen sulfide (H_2S), a toxic gas that poses significant risks to fish health and has been the underlying cause of mass mortality events in RAS facilities operating under saline conditions. Although optimization of system design can alleviate the potential risk of H_2S in production facilities, there are currently no efficient strategies in place that can be applied with a short response time for the removal of acute levels of H_2S. Therefore, to explore a possible chemical treatment for H_2S removal, we examined the kinetics of two common oxidizing compounds applied in RAS facilities; oxygen (O_2) and hydrogen peroxide (H_2O_2), and evaluate their applicability and efficacy in removing H_2S. Additionally, we tested whether nitrate (NO_3^-) acts as an oxygen donor in the chemical oxidation of H_2S.

Baseline oxidation rates and obtained half-lives ($t_{½}$) were considerably faster in clean seawater (SW, 118.5 ± 28.6 min) compared to process water from an industrial RAS (RASW, 168.0 ± 18.7 min). To evaluate the feasibility and factors influencing the oxidation rate of H_2S by H_2O_2, three different mole ratios of H_2O_2 to H_2S were tested. The addition of H_2O_2 to H_2S at a molar ratio of 1:1 had a significant effect on the removal rate and decreased $t_{½}$ of H_2S in SW and RASW by 49% and 75% respectively, the differences emphasizing the influence of organic matter on the required dosage for H_2S removal. Further increasing the H_2O_2: H_2S ratios to 2:1 and 4:1 in SW, $t_{½}$ was reduced to 21.7 ± 5.2 and 17.4 ± 6.1 min, respectively. NO_3^- was not found to be involved in the chemical removal of H_2S.

The findings presented highlight the importance of understanding the influence of water chemistry and quality on hydrogen sulfide removal kinetics, to implement effective strategies to alleviate the effects of acute H_2S events.

* *juhala@aqua.dtu.dk*

Microbial based early-warning tool for H_2S occurrence in RAS - A demonstration of principle

Deni Ribičić[1*], Shruti Gupta[2], Vebjørn Ulvang[2], Roman Netzer[1]

[1]SINTEF Ocean AS, Norway, [2]LetSea As, Norway

Abstract

Microbial communities play a crucial role in the functioning and stability of recirculating aquaculture systems (RAS). In this study, we investigated the temporal dynamics of microbial communities in two RAS units over an extended, but acute exposure period to hydrogen sulfide (H_2S), a notorious silent killer in RAS. We hypothesize that the presence of H_2S will act as a stimulus for the microbial community, particularly for microbes involved in sulfide metabolism, which could be leveraged for prediction and the development of an early warning tool.

By employing high-throughput sequencing of the 16S rRNA gene, we analyzed the composition and functional potential of microbial communities in both tank water and biofilter water samples. Our results revealed significant temporal changes in the microbial community structure within each RAS, indicating the adaptability of the microbial communities to changing conditions. Notably, the introduction of H_2S led to distinct microbial community shifts in the exposure RAS, which exhibited a larger spread over time compared to the control RAS. Machine learning (ML) model based on Random Forest algorithm was developed to predict the presence of H_2S based on microbial community structure, with high accuracy and specificity demonstrated in the hold-out dataset. Upon further analysis, specific taxa were identified as significant contributors to the model's performance, with *Planktotalea*, *Thiotrix*, *Devosia*, *Granulosicoccus*, *Albirhodobacter*, *Defluviimonas*, *Fusibacter*, and *Limnobacter* standing out. These taxa have known associations with sulfide and sulfur metabolism, suggesting their potential metabolic role in H_2S dynamics within the RAS environment, as initially hypothesized.

This study offers significant insights into the temporal dynamics of microbial communities in RAS and their response to H_2S exposure. The findings lay a strong foundation for the development of microbial-based monitoring and management strategies, particularly through the implementation of ML-based early warning tools. Such tools could prove highly relevant for industrial applications, enabling real-time water quality control in RAS and aiding in the timely detection and mitigation of potential issues related to H_2S and other microbial processes.

* *deni.ribicic@sintef.no*

Cysteine-degrading bacteria producing hydrogen sulfide in RAS

Alexandre Nguyen-tiêt[1*], Fernando Puente-Sánchez[2], Stefan Bertilsson[2], Sanni L. Aalto[1]

[1]DTU Aqua, Hirtshals, Denmark; [2]SLU, Upssala, Sweden

Abstract

Uncontrolled production of hydrogen sulfide (H_2S) is a major challenge in marine land-based RAS, as even very low H_2S concentrations can lead to massive fish mortality and significant economic losses. This H_2S production has been considered to originate from the activity of sulfate reducing bacteria, which would benefit from the high sulfate and organic matter present in marine RAS. However, it has been observed that H_2S production in marine fish organic waste is not always related to the abundance or activity of sulfate reducing bacteria, leading us to suggest that there is another microbial group contributing to H_2S production in RAS. One potential H2S source is sulfur-rich amino acids (cysteine, methionine) present in uneaten fish food and feces, that bacteria carrying specific genes could degrade it into pyruvate, ammonia, and H_2S under both anaerobic and aerobic conditions.

In this study, we examined the role of cysteine degradation pathway for H_2S production in different compartments of marine RAS (biofilter backwash (BW), activated sludge (AS), and biofilter biofilms (BF)). In anaerobic mixed reactors with cysteine as the only sulfur source, H_2S concentrations reached 51,37-82,05 mg H_2S/L already after 5 days, the H_2S production rate being 9.04 mg H_2S/L/h. While for the reactors with sulfate, the start-up of H_2S production took 10 days, and concentration above 50 mg H_2S/L was reached only after 14-28 days. The production rate was also lower in sulfate condition with 6.28 mg H_2S/L/h.

Using amplicon and metagenomic sequencing, we recognized three bacterial families that were more abundant in the cysteine reactors than in the sulfate reactors: *Fusobacteriaceae, Dethiosulfatibacteraceae,* and *Vibrionaceae*. These groups were verified to carry genes for cysteine degradation, indicating them to be responsible for high and fast H_2S production observed in RAS samples. We then analyzed in situ microbial communities in different marine RAS compartments and found these potential cysteine degrading H_2S producers to be present and to be even more abundant than the traditional sulfate reducing H_2S producers.

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* *alng@aqua.dtu.dk*

Notes

Nordic Co-operation

BioMar

6^{th} NordicRAS Workshop on Recirculating Aquaculture Systems

Tromsø, Norway, 3-4 October 2023

Day 1: Tuesday 3 October 2023

08^{00}-09^{30}	Registration
09^{30}-09^{55}	Opening
09^{55}-11^{00}	Session I: RAS in the Nordic Periphery
11^{00}-11^{30}	Coffee break
11^{30}-13^{05}	Session II: New approaches in RAS
13^{05}-14^{00}	Lunch
14^{00}-15^{20}	Session III: Water quality in RAS
15^{20}-15^{50}	Coffee break
15^{50}-17^{40}	Session IV: Ongrowing in RAS - experiences & challenges
20^{00}-23^{00}	Workshop dinner

Day 2: Wednesday 4 October 2023

09^{00}-10^{45}	Session V: Diseases and welfare in RAS
10^{45}-11^{15}	Coffee break
11^{15}-13^{00}	Session VI: H_2S and geosmine in RAS
13^{00}-14^{00}	Lunchnordic

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