DTU Aqua
National Institute of Aquatic Resources

5th NordicRAS Workshop on Recirculating Aquaculture Systems. Berlin, Germany, 7-8 October 2019

Book of Abstracts

By Johanne Dalsgaard (ed.)

DTU Aqua Report no. 350-2019

5th NordicRAS Workshop on Recirculating Aquaculture Systems. Berlin, Germany, 7-8 October 2019

Book of Abstracts

By Johanne Dalsgaard (ed.)

DTU Aqua Report No. 350-2019

The workshop is supported by:
AG-Fisk/Nordic Council of Ministers

Main sponsor of the workshop:
BioMar A/S

Colophon

Title:	5th NordicRAS Workshop on Recirculating Aquaculture Systems Berlin, Germany, 7-8 October 2019 Book of Abstracts.
Author:	Johanne Dalsgaard (ed.)
DTU Aqua Report No.:	350-2019
Year:	October 2019
Reference:	Dalsgaard, J. (ed.), 2019. 5th NordicRAS Workshop on Recirculating Aquaculture Systems. Berlin, Germany, 7-8 October 2019. Book of Abstracts. DTU Aqua Report No. 350-2019. National Institute of Aquatic Resources, Technical University of Denmark, 50 pp.
Cover photo:	Atlantic Sapphire, Homestead, USA. Photographer: Thue Holm.
Published by:	National Institute of Aquatic Resources, Willemoesvej 2, 9850 Hirtshals, Denmark
Download:	www.aqua.dtu.dk/publikationer
ISSN:	1395-8216
ISBN:	ISBN 978-87-7481-273-9 (web) ISBN 978-87-7481-272-2 (print)

Committee members

NordicRAS Network steering committee members

Helgi Thorarensen	*Holar University College, Iceland*
Jouni Vielma	*Natural Resources Institute Finland, Finland*
Per Bovbjerg Pedersen	*Technical University of Denmark, DTU Aqua, Denmark*
Torsten E.I. Wik	*Chalmers University of Technology, Sweden*

Organizing committee members for the NordicRAS Workshop

Johanne Dalsgaard	*DTU Aqua, Technical University of Denmark, Denmark*
Grete Solveig Byg	*DTU Aqua, Technical University of Denmark, Denmark*
Lars-Flemming Pedersen	*DTU Aqua, Technical University of Denmark, Denmark*
Per Bovbjerg Pedersen	*DTU Aqua, Technical University of Denmark, Denmark*

Correspondence:
Johanne Dalsgaard
jtd@aqua.dtu.dk

Contents

Welcome to the 5th NordicRAS Workshop

In 2011, we had the first NordicRAS workshop and pioneering Langsand Laks was at that time the first marine RAS to produce salmon full-cycle on land from eggs in FW to harvest in SW. Today - only eight years later - «every week, it seems that a new land-based project is announced» (https://salmonbusiness.com, 9 May 2019). The industry is expanding exponentially and it can be difficult to grasp the scale and speed of development.

During the next 1½ days, we will be presented to some of the large RAS facilities around the world going from North America to China (session I: RAS around the world). We will hear how far leading fish feed companies have come in developing feed specifically designed for RAS. This topic is essential since roughly speaking if it was not for the feed we would have no particle -and water quality issues in RAS (but of course also no fish production ☺). Session II will, in addition to addressing fish feed, discuss effects of suspended particles and present potential new means to remove them. Microbial water quality is a future focus area in RAS operation and session III will address some of the newest findings. Session IV has been entitled «RAS operations - experiences & challenges» but it might as well have been called «I know what I'm talking about because I'm there when it happens». Systems managers will talk about hands-on experiences with operating large scale RAS and challenges with implementing new technologies.

In 2014 and 2018, fish health specialists from mainly Finland, Denmark, and Norway organized a scientific workshop on diseases and fish health issues in RAS. Diseases, fish health, fish feed, micro particles, bacteria, and RAS management is tightly interlinked and we therefore aim at uniting forces in NordicRAS workshops to come. We pre-launch the collaboration this year in session V entitled «Fish health and water quality in RAS» and chaired by members of the fish disease specialist group.

Finally, session VI will get back to relevant microbiology issues addressing both harmful (H_2S and geosmin) and helpful (denitrification) microbial processes in RAS.

The interest in the workshop has been overwhelming. We have set a limit of 300 participants to keep some intimacy and to stick to the concept of all presentations made in the same room, but many more would have liked to participate. Similarly, we have had to reject many abstracts receiving many more than we have slots for. We are sorry for all rejections made and will consider how to address this in future workshops should it become a recurrent issue.

Some of the interest in this year's workshop undoubtedly comes from organizing it back-to-back with AE2019, and hoping for mutual benefits we thank European Aquaculture Society for their request and initiative.

Finally, we are very grateful to BioMar for once again being 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 5th NordicRAS and some hopefully interesting, "hyggelige", and rewarding days in Berlin.

Johanne Dalsgaard


BioMar
ORBIT
Innovating aquaculture with the world´s first dedicated feed concept for recirculating aquaculture systems
www.biomar.com
LET'S INNOVATE AQUACULTURE

Program

Session III: Microbial water quality in RAS
Chair: O. Vadstein, NTNU, Norway
Co-chair: S. Aalto, University of Jyväskylä, Jyväskylä, Finland

Session IV: RAS operations - experiences & challenges
Chair: J. Davidson, The Conservation Fund's Freshwater Institute, USA -
Co-chair: P.B. Pedersen, DTU Aqua, Denmark

Abstracts of oral presentations

Presented at the

5th NordicRAS Workshop on

Recirculating Aquaculture Systems

Berlin, Germany

7-8 October 2019

Miami Bluehouse, Homestead: current status and future plans

Thue Holm*

Atlantic Sapphire, Miami, USA

Abstract

"Atlantic Sapphire is well into the construction of the Miami version of their Bluehouse – an all-in-one aquaculture production facility that houses every stage, from hatching broodstock to processing of the harvest…The company is preparing for an increased demand for salmon. It plans to exponentially expand the Miami Bluehouse in size and scope as it moves through different phases. Phase one is due for completion by the yearend [2018] and will see its first harvest – 9,484 metric tons head-on and gutted (MT HOG) salmon – by the second quarter of 2020. Phase two will add 20,000 MT HOG by 2023. Phase three will add another 60,000 MT, for a total production of nearly 90,000 MT HOG, by 2026. The initial 384,000-square-foot facility in phase one will grow to four million square feet by phase three. The 100 direct jobs and economic impact equivalent of 2,700 jobs of phase one is predicted to grow to 21,000 indirect jobs by phase three."
(www.aquaculturenorthamerica.com/american-dream-within-reach-for-atlantic-sapphire-2000/).

thue@atlanticsapphire.com

RAS in Norway, Scotland and Canada: challenges, experiences and future plans

Trond Rosten*

Mowi ASA, Norway

Abstract

"Mowi ASA, formerly known as Marine Harvest ASA (until January 1, 2019)… is a Norwegian seafood company with operations in a number of countries around the world. The company's primary interest is the production, processing and sale of farmed salmon, the operations of which are focused on Norway, Scotland, Canada, the Faroe Islands, Ireland and Chile. The group has a share of between 25 and 30% of the global salmon and trout market… making it the world's largest company in the sector… Mowi also owns a 'value added processing' unit, which prepares and distributes a range of seafood products, and a number of smaller divisions.

The company assumed its current form as a result of massive expansion in 2006, when Pan Fish ASA conducted an effective three-way merger with Marine Harvest N.V. and Fjord Seafood…" (https://en.wikipedia.org/wiki/Mowi).

**Trond.Rosten@mowi.com*

Fredrikstad and Belfast: current activities and plans for RAS farming

Simon Dunn*

Nordic Aquafarms AS

Abstract

"Nordic Aquafarms is the first company in Norway to move salmon production into large-scale land-based systems. Our Fredrikstad Seafoods facility under construction is the largest such facility in Europe… The facility design will maintain stable water temperatures and high water quality year-round to enable a quality product. All discharge from the facility will be continuously cleaned and use of chemicals will be minimal to ensure a green footprint. The facility will facilitate grow-out of Atlantic Salmon to 3,5-4 kilos. Fresh gutted salmon will be delivered on a weekly basis to customers. Fully expanded, the facility will produce up to 6,000 tons of salmon annually."

Nordic Aquafarms INC was incorporated in Delaware in 2017 as the legal entity for our US business operations. Nordic Aquafarms has announced a large salmon farm in Belfast, Maine that will be under development in 2018 and beyond. The ultimate potential for this farm is estimated to 33,000 tons annual production." (http://www.nordicaquafarms.com/business-units/)

** sdd@nordicaquafarms.com*

RAS in China

Zhitao Huang[1]*, Ying Liu[2]

[1]Ocean University of China, Qingdao, China; [2]Dalian Ocean University, Dalian, China

Abstract

Aquaculture is an important component of fishery industry. The aquaculture production is 46.8 million tones in China in 2017, which accounts for the 58% production of the world. As an intensive form of aquaculture designed to maintain good water quality and provide safe and aquatic healthy products, RAS is becoming more and more popular in the past 30 years in China.

This presentation will focus on the development status, challenges, and strategies for future perspective of RAS in China.

**huangzt@ouc.edu.cn*

Feed for advanced aquaculture technology

Kyla M. Zatti[1]*, Kim S. Ekmann[2], Pedro G. Requeni[2]

[1]BioMar AS, Trondheim, Norway; [2]BioMar A/S, Brande, Denmark

Abstract

Efficient commercial RAS diets are typically characterized by three strong main pillars:

- High nutrient density and strict balance of essential nutrients
- High physical pellet quality
- Stabilization of fish faeces which allows for easy removal from recirculation water

Feed pellet quality and faecal stability are of outmost importance in modern commercial intensive RAS systems which strive for maximum fish growth and feed utilization. The complexity of optimizing both pellet and faecal quality can be daunting with ever changing raw materials (RM), diet formulations and processing parameters. In a RAS system, the main components to be removed and broken down are faeces and break-down components from metabolism. However, feed waste and feed pellet disintegration are often neglected. Ideally, feed waste is virtually zero, pellet quality is optimal and therefore, system loads on filters are negligible. However, in many commercial RAS units fish growth is so highly prioritized that a small, but significant, feed waste is expected. This feeding strategy requires a very rapid and efficient removal of wasted feed pellets to avoid feed pellet disintegration as much as possible.

Shifts in the bacterial balance of both the water and the biofilters creates instability within the system. Disintegrating feed pellets, in the RAS system, provides the perfect media for opportunistic bacteria and autotrophic bacteria to bloom, which outcompetes the nitrifying bacteria within the biofilter; negatively impacting the whole systems microbial balance and reducing the biofilters capacity. In addition, this can lead to gill irritation and inflammation reducing the overall performance of the fish. This study focused on improving fish faeces and pellet stability by manipulating dietary RM composition and optimizing the diet using a scoring system. The model can be applied to several species in both fresh- and sea water RAS. Several different faecal and pellet stability evaluation parameters were employed in a model which allows for the prediction and active dietary optimization of RAS diets. Its main purpose for faecal stability focuses around particle size distribution analyses, visual scoring, ease of precipitation and nutrient leaching. For pellet quality the model focuses on pellet strength, durability, oil leakage, sinking speed and water holding capacity. Each parameter is individually scored on a raw material level; first being exposed to fractal analysis and secondly to a criteria, options, weight and score (COWS) analysis with the resulting scores being introduced into a commercial least-cost diet optimization program.

The results showed large differences in RAS diet suitability between raw materials commonly used for feed production, which in turn emphasizes the need for active manipulation of RM composition when optimizing commercial RAS diets. In order to ensure that RAS feed customers can optimize fish growth without jeopardizing the water quality of the system, it is essential to first optimize pellet quality by subjecting finished feeds to several physical pellet quality tests and to ensure the most optimal combination of RMs is in the diet to support faecal stability.

kylza@biomar.com

Development of feeds for recirculating aquaculture systems

Ingunn Stubhaug[1]*, Paulo Fernandes[1], May-Helen Holme[1], Alex Obach[1]

[1]Skretting Aquaculture Research Centre, Stavanger, Norway

Abstract

Growing fish in recirculating aquaculture systems (RAS) opens up for many new opportunities and markets. However, these systems present several challenges for rearing the fish. The aquaculture industry is estimating approximately 500 000 metric tons of new planned production capacity of salmon in recirculated systems by 2026. This leads to an increased demand for optimised, high quality feeds for RAS.

In RAS, waste nutrients and particles originating from fish and feed need to be continuously removed from, or transformed within, the system. This requires different properties of the feed compared to open sea cages where excess nutrients are rapidly diluted by the sea and removed from fish enclosures by the water current. Feeds for RAS need to be of remarkably high physical quality, made from high quality raw materials, with optimised digestibility of macro- and micro-nutrients. In addition, it must result in highly stable faeces.

Obtaining a faecal quality that increases removal efficiency from the system and has minimal dry matter loss in the water column has been a cornerstone in our research for many years. This has led to lower mineral and nutrient load to the system, thereby improving the stability of the biofilters. Recently, there has been an increased focus on raw material quality and usage in addition to digestible protein and energy. A summary of the latest research and development on feeds for recirculating systems will be presented and discussed.

**Ingunn.Stubhaug@skretting.com*

Feed designed for RAS

Robert Tillner[1]*, Florian Nagel[1], Hanno Slawski[1]

[1]Aller Aqua Research Gmbh, Buesum, Germany

Abstract

Feed is the most influential external factor in RAS and feed manufacturers need to provide the following benefits from a farmers' point of view: high feed efficiency, optimal water quality and consequently high fish growth.

To meet the high standards in RAS and create benefits for RAS farmers, several features of feeds can be targeted and optimised. The feeds must be highly digestible and palatable to secure a high transfer of nutrients into the fish body for metabolism and growth and reduce nutrient losses into the water. Another important factor is the quality of the faeces. Low stability and weight of faeces dramatically reduces the efficient removal by mechanical cleaning units, such as drum filters in RAS. As small faeces particles become smaller and more and more suspended, they are not removed by the drum filter but accumulate in the water and are carried into the biofilter. Balancing nutrient digestibility of raw materials and faeces quality to ensure shaped and compact faeces particles will allow effective removal and the lowest possible impact of suspended matter on fish environment and filtration technology. Both nutrient digestibility and faeces quality are applied to determine the exact amount of nutrients required by fish of different life stages for optimal growth and the optimal ratio of digestible protein to digestible energy (DP:DE). The optimal ratio between digestible protein and digestible energy allows for minimal waste of feed protein, mainly in the form of ammonia and urea, and at the same time allows the optimal usage of precious feed protein for fish growth.

In addition, the feed pellets must match the requirements of RAS in terms of low dust content, low leaching of nutrients, high stability and optimal density. Taken together, these features result in high and constant performance.

**rt@aller-aqua.com*

A re-evaluation of issues associated with suspended solids in salmonid aquaculture

Mark Schumann[1,2]*, Alexander Brinker[1,2]

[1]Fisheries Research Station of Baden-Württemberg, Langenargen, Germany; [2]Limnological Institute, University of Konstanz, Konstanz, Germany

Abstract

Total suspended solids (TSS) in aquaculture of salmonids are associated with a number of issues ranging from direct physiological damages of farmed fish up to indirect effects like endangering system stability or providing substrate for bacterial growth. Several limit values of TSS in salmonid aquaculture systems appear in literature without an adequate scientific basis or without respect to transferability, as e.g. the system-related differences of solid composition are not considered.

A meta-analysis was conducted to work out the variations of chemical composition and physicochemical properties of suspended particles in the different system types of salmonid aquaculture. Additionally, solid loads characteristics like particle size distribution or concentration were linked to fish physiology and farm operations.

The results give an overview about the development and properties of suspended solid concentrations in common farming systems for salmonids under different production intensities. As expected, RAS systems show the highest average concentrations of suspended solids, including fines, however, the collected data do not support the common perception of a positive correlation between production intensity and suspended solid load in RAS. Overall, the data suggests that the discrete impact of solid load for salmonid species is less of concern, but coupled and indirect effects are the probable drivers for issues attributed to particle-associated causes. Cleary, research is needed on the interaction effects of solids and other water parameters as the issue is multifactorial and the interplay needs to be better understood.

**Mark.Schumann@lazbw.bwl.de*

Particle removal by separators including fluidized lamella packings – An alternative for screen filtration in small-scale RAS?

Andreas Müller-Belecke[1]*, Andreas Spranger[2], Dominik Dirnbach[3], Sören Knoll[3]

[1]Institute of Inland Fisheries Potsdam-Sacrow, Potsdam, Germany; [2]Kunststoff-Spranger GmbH, Plauen, Germany; [3]HTWG Konstanz, Konstanz, Germany

Abstract

Especially in smaller RAS, which have a high potential for research issues and in the context with regional production concepts including direct marketing, drum filters belong to the system components, demanding for the highest investments and lead to comparably high backwash water losses. It is well known that stagnating biocarrier packings of inadequately operating moving bed biofilm reactors (MBBR) have positive short term effects on particle removal. The aim of the present study was the development and test of a structurally simple and cost-effective device for particle removal in RAS. The tested separator device combined the functionality of a vertical flow, round settling tank including a cleaner arm with the advantages of a lamella separator, by incorporating fluidized biocarrier packings.

The separator equipped with different fluidized biocarrier packings was consecutively performance tested for two month periods in comparison with the drum filter HDF 801 1G (60 µm screen) in a small scale RAS (8 m³ productive volume). The RAS was stocked with pikeperch close to marketing size (ca. 50 kg m^{-3}; ca. 2.5 kg of administered feed per day). In addition to growth- and water quality parameters, discharge water generation, hydraulic retention times, suspended solids, COD and turbidity were monitored in the in- and outlets of the particle removal devices.

The packings specific surface area positively correlated with particle removal efficiency. But as expected, fine textured packings tended to clog too fast and enduring for a low-maintenance operation. Equipped with a 400mm strong fluidized packing of 50mm Pall-Rings, at a hydraulic retention time of 12 min., the removal of suspended solids by the separator was higher (23.8% ± 11.4%; mean value ± standard deviation) compared to the drum filter (13.8% ± 9.6%) and clogging of the packing could easily be prevented by manual stirring for some seconds twice a day.

The developed separator with fluidized lamella packing proved to be a suitable mechanical purification device for warm water RAS at low to moderate recirculation rates. Corresponding separators require low investments, are easy to construct, to handle with manageable maintenance costs and possess a low risk for malfunction. They allow for the design of RAS with low head losses and minimum fresh water requirements for particle removal. Applicability in cold water RAS is not given as here sewage fungus may clog even roughly textured lamella packings.

Acknowledgements: This research was funded by the German Federal Ministry of Food and Agriculture (BMEL).

**andreas.mueller-belecke@ifb-potsdam.de*

Protein skimmer and ozone in RAS: valuable tools for clear water production

Jaime Orellana[1]*, Bert Wecker[2], Uwe Waller[3]

[1]Erwin Sander GmbH, Uetze-Eltze, Germany; [2]neomar GmbH, Uetze-Eltze, Germany; [3]Hochschule für Technik und Wirtschaft des Saarlandes HTW, Saarbrücken, Germany

Abstract

In RAS systems, metabolism products are removed from the water by mechanical, chemical and biological filter stages. Water quality, especially in terms of turbidity, are significant factors for the rearing of tasty, high-quality fish. In contrast to conventional RAS, modern designs allow the removal of very fine solids. This is achieved by a so-called two-step solid separation. Investigations over the past 20 years, have proven that a combination of mechanical separation and boundary surface filtration, will achieve clear water rearing conditions. In the mechanical separation (drum filter) coarse solids are filtered from the process water. Extremely small particles are removed by means of foam fractionation in a so-called protein skimmer. Fine air bubbles are used as filter material by aggregating active substances, such as protein compounds, with fine particles and microorganism.

Furthermore, to enhance the effectiveness of the foaming process, ozone is mixed into the fine bubbles. By dosing the amount of ozone inside the skimmer, the majority of bacteria and facultative pathogens is inactivated. At the same time, biologically non-degradable substances (*e.g.* geosmine and 2-methylisoborneol) that caused the water to become increasingly yellow and turbid, and affect the taste of fish negatively, will be oxidised.

The control of the ozone dosage is carried out via the redox potential value (ORP). The term “ozone oxidation” expressed in millivolts (mV) will in this case used to denominate any reaction in which ozone and free radicals, originated in a chain reaction, transforms chemical compounds, and thereby raised the ORP value. From the operational point of view, experiences over two decades shows that the ozone amount to be used in RAS it’s not an operator input, but a water quality demand. This means that, from the design philosophy behind the RAS conception, up to the species reared, and the type of the feed fed, all this will affect the water quality and decide the quantity of ozone that will be needed to achieve the wanted product quality.

Farm managers in marine, brackish, and fresh water system, can profit from clear water production systems in terms of increasing both, mean stocking density without affecting fish welfare, and survival rate due to a better water quality. This will also drive to a better feed intake and utilisation.

**jaime.orellana@aqua-sander.de*

Combining UV and micro filtration to manage microbial water quality in recirculating aqua culture systems

Kim João de Jesus Gregersen[1*], Per Bovbjerg Pedersen[1], Lars-Flemming Pedersen[1], Dibo Liu[1], Johanne Dalsgaard[1]

[1]DTU Aqua, Technical University of Denmark, Hirtshals, Denmark

Abstract

Recent studies have focused on micro particle build-up in recirculation aquaculture systems (RAS), and a correlation between micro particles and microbial activity has been shown. The lower size spectra of what is commonly referred as micro particles is presumably composed of free swimming bacteria and therefore, the use of micro filtration and UV targeting bacteria should result in a reduction in bacteria (both free-swimming and surface-attached) and an overall reduction in micro particles (including bacteria) present in the system.

This study evaluated how micro particle build-up and microbial activity are affected by UV irradiation and micro filtration. Using 12 identical pilot scale RAS stocked with rainbow trout (*Oncorhynchus mykiss*), a two-factor factorial experiment was carried out testing in triplicate systems the effect of systems with or without UV irradiation in combination with cartridge filtration (1 or 200 µm pore size) on selected water quality parameters. The trial ran for 13 weeks and showed that both main factors (UV and cartridge filtration) had significant effect on micro particle distribution and microbial activity in the systems.

By the end of the trial, UV treated systems were significantly lower in micro particle numbers, micro particle surface area, dissolved COD, and microbial activity compared to systems without UV and independently of cartridge filtration pore size. UV thus appeared to reduce micro particles largely by destroying bacteria. With respect to micro filtration, a 1 vs. 200 µm pore size significantly reduced the number of micro particles, micro particle volume, and micro particle surface area independently of UV treatment. This was accompanied by a significant reduction in particulate COD and microbial activity. Hence, cartridge filtration appeared to prevent a build-up of micro particle by directly removing bacteria and bacteria substrate.

In conclusion, the study demonstrated that combining UV and particle removal is a potential way of managing microbial water quality in RAS. Furthermore, the results seem to sustain that a large proportion of micro particles are in fact living bacteria.

Acknowledgements: The study was partly funded by the European Maritime and Fisheries Fund (EMFF) and the Danish Ministry of Food, Agriculture and Fisheries as part of project ”Vet-løsninger” (J.no. 33111-I-16-029).

*jdjg@aqua.dtu.dk

Highly standardised flow cytometry as a flexible tool for realtime monitoring of microbial water quality

Michael D. Besmer[1]*, Konstanze Schiessl[1], Frederik Hammes[2]

[1]onCyt Microbiology AG, Zürich, Switzerland; [2]Department of Environmental Microbiology, Eawag, Dübendorf, Switzerland

Abstract

Most microorganisms in aquaculture are harmless but occasionally they can have negative effects on water quality or the infrastructure itself. On the other hand, bacteria and algae can also keep each other in balance and are even used in production (algae for feed) and treatment (bacteria in biofiltration) processes. Hence, it is crucial to closely monitor these organisms to decide how they need to be managed in a given system/process.

As microbial dynamics often occur at short timescales (seconds to days), their tracking requires sampling and analysis at very short intervals and in real-time allowing for immediate interpretation/reaction. For decades this was impossible with conventional, cultivation-based methods but also with advanced molecular methods. They are all still too labour-intensive, time-consuming, and costly.

One alternative approach is flow cytometry – a detection method adapted from medicine with specific advantages: rapidness, sensitivity, reproducibility, accuracy in quantification, and differentiation of total and intact cells. Through radical standardisation of sample preparation, instrument settings, and data analysis, an originally complex detection method is now fully applicable to water quality monitoring. Time to result is 15 minutes and analysis is straightforward with minimal training and clear SOPs. Furthermore, we have developed a fully automated online flow cytometry system that overcomes the restricting practice of grab-sampling and subsequent cultivation on agar plates (Besmer *et al.*, 2014). In short, water samples are drawn directly from the water resource of interest every 15 min, mixed with a fluorescent stain, incubated, and then measured with the flow cytometer. Rinsing and extended cleaning are performed regularly.

Flow cytometry can detect all bacteria (including viable but non-culturable) and assess viability (e.g., for disinfection processes based on oxidation and/or heat). Over the last years we discovered a myriad of interesting and often unexpected microbial dynamics at time scales from minutes to weeks and bacterial concentrations levels between 104 and 107 cells ml^{-1}. For example, clear diurnal fluctuations in bacterial concentrations were linked to photosynthesis in rivers, to intermittent water extraction in groundwater, to varying water production rates in a drinking water treatment plant, and to daily water usage in a building. In addition, the influence of precipitation events and increased discharge on bacterial concentrations was accurately quantified in rivers, springs, and groundwater.

These results boost the idea of advanced monitoring of microbial dynamics, which is critical for a better understanding of underlying causes of fluctuations as well as the ecological and operational consequences thereof. While originally applied for bacteria in drinking water, applications of both fresh- and saltwater are in progress looking at bacterial and algae concentrations and viability in parallel. We expect that these findings will massively stimulate improvement of process monitoring, water treatment design and improvement (e.g., disinfection in RAS/CSS, ponds), optimisation of feed and other production processes, and conceptual approaches to smarter sampling schemes, but also open up new applied and fundamental research directions.

**michael.besmer@oncyt.com*

Microbial water quality – assessment of baselines and application perspectives

Pedersen, P.B.[1]*, Dalsgaard, J.[1], Pedersen L-F.[1], Huang Z[2]

[1]DTU Aqua, Technical University of Denmark, Hirtshals, Denmark; [2]Ocean University of China, Qingdao, China

Abstract

To apply and operationalize the concept of microbial water quality and to be able to determine whether conditions are acceptable and within normal ranges baseline studies from commercial farms are needed. To establish such a baseline an intensive saltwater RAS producing Atlantic Salmon (*Salmo salar*) was studied for six weeks with sampling campaigns twice a week (12 samplings in total). Two separate grow-out units were studied with eight sampling points in one of them ($biofilter_{in}$ $biofilter1_{out}$ $biofilter2_{out}$ $skimmer_{in}$ $skimmer_{out}$ $3xtank_{out}$) and three sampling points ($tank_{in}$ and $2xtank_{out}$) in the other. Measurements included assessment of bacterial activity (H_2O_2-degradation rate, Bactiquant®), number of bacteria as well as live/dead bacteria (flow cytometer), number and surface area of particles (Coulter Counter), as well as COD and BOD_5. On system level temperature, pH, DO, and turbidity was measured before each sampling campaign.

Based on the study, baseline levels and normal variation therein has now been established for several relevant parameters considered important for the assessment of microbial water quality for one specific SW-RAS. Similar studies on a number of commercial RAS are needed to describe overall baseline levels on a global perspective, presumeably with values and ranges being specific for different types of RAS (intensity, salinity, design, etc).

In addition to the establishment of baseline levels, interesting results were observed within systems, underlining the effect of individual components of a RAS. These results will be presented and discussed.

Finally, differences was also observed between the two grow-out units, each being a separate RAS. The results indicate that the measurements made could be used to describe stable and "safe-margin" operation of RAS as well as potential indicators of instability and system overload.

The potential application and possibilities to support commercial operation in RAS will be discussed.

**pbp@aqua.dtu.dk*

Next generation monitoring of biological water quality in RAS

Deni Ribičić[1]*, Stine W. Dahle[1], Roman Netzer[1]

[1]SINTEF Ocean, Trondheim, Norway

Abstract

The microbial communities, their functions and interactions in recirculating aquaculture system (RAS) are crucial for the biological water quality and play thus an important role for the health and fitness of the cultured fish. Certain microbial assemblages may impact fish health positively, while the others may exhibit sever negative effects, or even cause mortality.

At present, strategies used for detection of microorganisms in RAS employ rather an atomistic approach, where methods are mainly limited to the detection of specific fish pathogens or other unwanted bacteria by low-coverage semi-quantitative real-time PCR (qPCR) or agar plating. This reveals an immense knowledge gap on microbiota compositions in such complex system as RAS. Consequently, there is an urgent need for up-to-date, fast and comprehensive techniques, favouring holistic approach for characterization of microbiota and their interactions with cultured species and amongst each other.

The emergence of next generation sequencing (NGS) technologies based on high-throughput sequencing in combination with advanced bioinformatics and deep machine learning have opened the door for deeper insight into structures, functions and interactions of microbial communities in complex environments. NGS allows the detection of tens of thousands of microbes within each sample, providing a snapshot of microbiota composition at any given time. Although NGS is frequently used to explore microbial communities in different environments, its application in aquaculture is still rare and only little information is available.

In our ongoing project MONMIC we have employed 16S rRNA gene amplicon NGS over a period of 15 months with bi-monthly sampling at five different Norwegian salmon farms and at four different locations within each RAS (inlet water, tank water, biofilm and biofilter). The project is of a pioneering character as it has resulted in a comprehensive dataset, accounting more than 1000 samples and covering a whole smolt production period. Such exhaustive information enabled us to identify core microbiota at different locations within and between RAS and to observe characteristic changes depending on environmental conditions. Obtained dataset represents a basis for future studies on microbiota in RAS, and it serves also as input for advanced modelling and machine learning (ML). Preliminary results show that ML algorithms trained on extensive and detailed datasets have a potential to be used as predictive tools for improved control of biological water quality that is critical for fish health, welfare and streamlined RAS production.

**Deni.Ribicic@sintef.no*

Assessment of bacterial activity in biofilm - examples and perspectives

Lars-Flemming Pedersen[1]*, Kim J.D.J. Gregersen[1], Per B. Pedersen[1], Peter V. Skov[1]

[1]DTU Aqua, Technical University of Denmark, Hirtshals, Denmark

Abstract

A number of challenges and investments in RAS are associated with control of bacteria. One of the consequences of increased feed loading and long retention times in RAS is favourable conditions for bacterial growth. This manifests in potentially deteriorated water quality, biofilm formation on unwanted surfaces such as trickling filters and inside pipes, and increases the load on biofilters.

The associated efforts to control bacteria include, among other, improved solids removal, management of biofilters, and physical and chemical disinfection of the process water. While new knowledge of microbial water quality has emerged by use of microbial assays and sequencing tools, several issues related to biofilm formation, dynamics, implications, and control options still remains to be addressed.

This presentation describes the mode of concept of new methods to assess bacterial activity in biofilm. Potential applied use of the methods to support industrial challenges related to water-biofilm interactions will be discussed.

**lfp@aqua.dtu.dk*

Salinity change strategies for biofilters in RAS for Atlantic salmon (Salmo salar)

Sharada Navada[1,2,*], Marianna Sebastianpillai[1*], Frédéric Gaumet[2], Olav Vadstein[1], Ragnhild Fossmark[1], Øyvind Mikkelsen[1], Jelena Kolarevic[3]

[1]NTNU, Norwegian University of Science and Technology, Trondheim, Norway; [2]Krüger Kaldnes AS (Veolia Water Technologies), Sandefjord, Norway; [3]Nofima AS, Tromsø, Norway

Abstract

The production of Atlantic salmon (*Salmo salar*) in recirculating aquaculture systems (RAS) is challenged by the requirement for varying salinities during the different growth stages of the fish. Salinity changes can adversely impact the nitrification rate in RAS biofilters, which is a vital part of the water treatment system for the conversion of ammonia to nitrate. As even low concentrations of ammonia and nitrite can be toxic to fish, it is necessary to develop a strategy to change the salinity in RAS with the least possible impact on nitrification efficiency. With this objective, we performed three studies in lab-scale moving bed biofilm reactors (MBBR).

In the first study, we compared the nitrification in MBBRs transferred from freshwater to seawater under different rates of daily salinity increase. At all the rates of salinity increase, the nitrification performance decreased by 50-90% upon transfer to seawater. Further, the ammonia removal was independent of the salinity increase rate, and dependent mainly on the salinity and the acclimatization time. In the second study, we evaluated the effect of seawater priming on fresh- and brackish water (12‰ salinity) MBBRs. Seawater priming significantly reduced the negative impact of salinity increase in freshwater MBBRs, while brackish water MBBRs were inherently robust to salinity increase. In the third study, we compared a parallel start-up of two full-scale MBBRs in fresh- and brackish water, respectively. At the end of two months, the ammonia oxidation capacities in both MBBRs were similar, whereas the nitrite oxidation capacity was higher in the freshwater MBBR than in brackish water MBBR. However, the rapid increase in nitrate concentration indicated that the nitrification process had started up in both the MBBRs.

In conclusion, the results of these studies show that seawater priming of freshwater biofilters or startup in brackish water are feasible strategies for biofilter management in RAS. These strategies can be instrumental in the shift of post-smolt production from net-pen farming to land-based RAS.

Acknowledgements: This project is a part of CtrlAQUA SFI, Center for research-based innovation funded by the Research Council of Norway and the Center partners, including Krüger Kaldnes AS (#237856/O30, #270888/O30).

**sharada.navada@krugerkaldnes.no; marianna_sebastianpillai@hotmail.com*

Increase in salinity as a driver for bacterial succession in RAS for Atlantic salmon (*Salmo salar*) smolt production

Ragnhild O. Fossmark[1*], Kristian Nordøy[2], Stine W. Dahle[3], Olav Vadstein[1], Stein W. Østerhus[1] and Kari J.K. Attramadal[1]

[1]Norwegian University of Science and Technology (NTNU), Trondheim, Norway; [2]Let Sea AS, Sandnessjøen, Norway; [3]Sintef, Trondheim, Norway

Abstract

Production of post-smolt in RAS is becoming more common in salmon farming. Longer production time on land yield more robust fish during sea transfer and reduce exposure time with sea lice and general tough conditions off-shore. From an operational perspective, the question of whether the post-smolts should be reared in freshwater or brackish/saltwater is discussed. The latter involves that water of higher salinity is introduced into the RAS, which constitutes some challenges. The biofilter function is especially vulnerable to such an environmental shift. Increased salinity will affect the microbial community dynamics of bacteria in the water and biofilter biofilm, and it is not known how these dynamics unfold. The objective of this experiment was to study how salinity affected nitrification kinetics, the general microbiota in RAS and how different operational regimes with salinity affected fish health and growth.

The experiment was carried out at Let Sea's RAS facility at Dønna, Nordland. It included two parallel commercial size RAS with Atlantic salmon (*Salmo salar)* smolt. One system operated at constant seawater (S) 28 ppt, and the other with a gradual increase in salinity from 3 – 25 ppt (BS) during a period of 30 days. Bacterial communities of water, biofilm and fish gut were characterized through Illumina sequencing. Nitrification capacity tests were performed on biofilter media from the BS system at different salinities. Ordination (PCoA) based on Bray-Curtis similarities showed that the gut of individual fish in BS had similar microbial community structures in the period with low salinity, and evolved to become more similar to communities in S-individuals as the salinity increased over 12 ppt. In water samples in BS, there was a shift in the bacterial communities in the period between 6 and 12 ppt. From 12 – 25 ppt the communities were similar and more stable. The nitrification efficiency decreased between 4 – 12 ppt, and TAN and NO_2 accumulated in the system. From 12 – 25 ppt, the biofilter seemed to deal well with the increased salinity and less accumulation was observed. Fish in the S system had higher mortality and higher growth than the fish in the BS-system. Salinity was shown to be a driver for bacterial succession in the system, and a threshold salinity of 12 ppt induced major changes in microbial community dynamics for both water, gut microbiota and nitrifying efficiency of the biofilters.

Acknowledgements: This study was funded by Let Sea AS, RFF NORD and SkatteFunn.

**ragnhild.o.fossmark@ntnu.no*

Hatchery system (RAS vs FT) effects on fish microbiota for Atlantic salmon, *Salmo salar*

Jeremiah J Minich[1]*, Khattapan Jantawongsri[2], John Bowman[2], Abigail Elizur[3], Stewart Fielder[4], Rob Knight[5,6], Eric Allen[1,5], Barbara Nowak[2]

[1]Marine Biology Research Division, Scripps Institute of Oceanography; [2]Institute for Marine and Antarctic studies, University of Tasmania, [3]Department of Biology, J. University of Sunshine Coast; [4]Department of Primary Industries, New South Whales; [5]Center for Microbiome Innovation, University of California San Diego; [6]Department of Pediatrics, University of California San Diego

Abstract

A concern for RAS systems over traditional flow-through (FT) is a negative impact on fish health which may be due to microbial dysbiosis to the fish or the environment (water). In this study we evaluated how the built environment of a hatchery is influenced by the hatchery type from three Atlantic salmon (*Salmo salar*) hatcheries (RAS n=2, FT n=1).

For Atl salmon, six fish (300 days post hatch) were sampled from three independent tanks in each of the three hatcheries for a total of 54 unique fish along with a tenth tank. Water and a swab of the tank side biofilm was collected from each of the nine tanks to evaluate the environmental microbiome. To assess the entire mucosal microbiome, the gill, skin, and digesta were swabbed from each fish and processed for 16S rRNA analysis using the EMP protocols.

The water and tank biofilm communities, especially from the RAS systems, had a higher microbial richness compared to the fish mucus. The three body sites each had unique microbial communities ($P<0.001$). Within each body site (gill, skin, and digesta), microbiomes were uniquely driven by the hatchery systems ($P<0.001$) with both RAS systems being more similar. Skin and digesta from RAS reared fish had about 2x the richness compared to FT reared fish. Comparing the tank system with the fish mucus, we found that both the water and tank biofilm richness was positively correlated with skin and digesta richness with the biofilm association being slightly stronger. Strikingly, the gill, skin and digesta communities were more similar to the origin tank biofilm vs. all other tanks suggesting that the tank biofilm has a direct influence on the fish communities. For water samples, the skin and digesta were more similar to the origin tank compared to all other tanks. Furthermore, the gill and skin communities were always more similar to tank water and tank biofilm than the digesta samples. Using histology, both digesta and skin microbiomes were associated with skin mucus cell levels indicating a direct tie to fish health.

The results from this study provide evidence for the first time an association and link between the tank microbiome and the fish microbiome. We show how the skin microbiome of the fish is most influenced by the environmental community which in turn is also related to skin mucus health.

**jjminich@ucsd.edu*

Savings in external carbon used for activated sludge denitrification of RAS aquaculture effluent using production sludge-COD as part of the carbon source

Clément Crouzet*[1], Arne Bækgaard[1], Gert Petersen[2], Knud Leth Nielsen[3], Kenneth Janning[4], Henrik Mortensen[5]

[1]Danish Salmon A/S, Hirtshals, Denmark; [2]Gert P Environmental, [3]Aquahouse, [4]E-Project, [5]CM AQUA

Abstract

At the Danish Salmon RAS facility in Hirtshals, Denmark the wastewater coming from the nitrified, filtered effluent discharge flow has been treated in a Pilot Plant for nitrogen removal since May 2019. The activated sludge volume of the Pilot is 195 m^3 and the surface area of the sedimentation tank is 45 m^2.

The basic process in the Pilot Plant is an activated sludge tank and a sedimentation tank using a traditional suspended solid process for the denitrification. The experiments are divided into two main experimental campaigns:

1. Conventional activated sludge denitrification using only external carbon source (acetate)
2. Denitrification assisted by hydrolysis of part of the production sludge-COD to save external carbon

At present the first campaign has ended and the experimental results from this part of the project has been processed and evaluated using hydraulic loads up to 35 m^3 process water/hour. These basic results for the campaign using only external carbon source will be presented as well as the experimental setup and background for the two campaigns and an estimate on the costs for a 1000 ton/year treatment unit.

The first experimental results are divided into hydraulic considerations on the maximum allowable hydraulic surface load of the sedimentation tank and the maximum loading and conversion capacities of the activated sludge.

Main results, first campaign:

Maximum hydraulic load to sedimentation:	0.8 m/hour
Maximum sludge load to sedimentation:	2.5 kg SS/m^2/hour
Maximum denitrification capacity:	5 g N/kg SS/hour
Maximum volumetric DN-capacity:	0.4 kg N/m^3 DN-tank/day
Process temperature:	14-15^oC

Acknowledgements: This research was funded by Danish Salmon, Aquahouse, CM AQUA and The Ministry of Environment and Food of Denmark through the MUDP programme: Miljøteknologisk Udviklings- og Demonstrationsprogram (MUDP).

clement@danishsalmon.dk

RAS experiences from Finnmark – solutions to the Arctic challenge

Bjørn Hovrud*

Laksefjord AS (Lerøy), Lebesby, Norway

Abstract
After a short introduction of Lerøy Seafood Group ASA and its northern salmon operation, I will speak about our experiences in general with RAS and a combined flow through and RAS facility.
I will then speak about the challenges of cage-based Arctic salmon operations and how brackish RAS can be used to overcome this challenge. Finally, I will speak about our «journey» from freshwater RAS via R&D projects on brackish RAS and test productions of postsmolt on brackish RAS, to dedicated large scale brackish RAS postsmolt production and the RAS design and production planning that has come out of this process.

**bjorn@laksefjord.as*

One year of harvest in the Swiss Alps

Thomas Hofmann*

Veolia Aquaculture Manager and former farm Manager of Swiss Alpine Fish AG

Abstract

4 years ago, Swiss Alpine Fish went ahead to build the first land based RAS2020 in the Swiss Alps to produce 600t of salmon for the local market. Over the last year, daily harvests have been taking place and 7 batches have now been successfully harvested from the farm.

Being one of the pioneers in land based RAS operations has taken us to learn and overcome some of the bottle necks this industry has been facing for a number of years.

Realistic bio-plan, reliable technology, mitigating risks while ultimately producing a top shelves quality salmon will be addressed to describe what has been done in this particular facility.

From egg to harvesting 4kg fish and further to processing and smoking on site, we will look into various lessons we have learnt during this exiting journey.

We will take a deep dive into the main challenges we had to overcome such as off flavor, optimal performance of the fish and moving large fish on land without altering their welfare or regulatory compliances.

**thomas.hofmann@veolia.com*

From RAS effluent to new intake water by woodchip bed, constructed wetland and sand infiltration treatment

Jouni Vielma[1]*, Tapio Kiuru[1], Petra Lindholm-Lehto[1], Jani Pulkkinen[1], Juha Koskela[1], Antti-Jussi Lindroos[1], Anna-Kaisa Ronkanen[2], Björn Klöve[2], Antti Pasanen[3], Lauri Solismaa[3], Muhammad Muniruzzaman[3]

[1]Natural Resources Institute Finland (Luke); [2]University of Oulu; [3]Geological Survey of Finland

Abstract

RAS discharge management is one of the key issues to develop the sector, since RAS does not automatically result in low nutrient emissions. Research has helped to develop discharge management systems such as wetlands and woodchip beds that have been adopted by commercial Danish model trout farms.

To further develop the Danish concept, we have modeled, carried out laboratory scale studies and built a system with annual capacity at appr. 14 tons. The concept includes treatment of RAS effluent by a hybrid solution of woodchip bed, constructed wetland and sand infiltration. By this three-step process, we are aiming to remove effluent nitrate, phosphorus and organic matter to acceptable levels, to re-use the water in the RAS process and to reduce need of fresh intake water.

In two lab studies on individual RAS, side-loops consisting of woodchip and sand filtration units were established. Control RAS without treatment system received lake water at 500 L/kg feed. In the first experiment, the treatment provided 250 or 400 L/kg of the replacement water thus decreasing the new water intake to 250 or 100 L/kg. Woodchip beds had 57 and 91 L of birch chip with hydraulic residence time (HRT) of 36 hour. The surface fed sand filter had effective porosity of 0.35, and 80 % of the height was water saturated conditions, with HRT of 1.2 days. Nitrate removal in the woodchip beds were insufficient, declining throughout the experiment, being at 9 g N/m3/day in the end of the 10 week experiment. In the second experiment, control RAS (new water 500 L/kg feed) and RAS with the treatment system (new water 100 L/kg feed) have been run for 29 weeks.

Based on the lab experiments, the field scale pilot system was built. Total volume of woodchip bed and sand filter are 50 and 650 m3, and CW has a surface area of 135 m2. The top most appr. 50 cm woodchip layer were planned to stay dry and to serve insulation for winter. Vertical flow CW was planted with common reed Local sand and gravel were used in all units which also have a bentonite layer as a bottom structure. Water volume and, thus, HRT in all units can be adjusted. Challenges in the operation of the system include e.g., finding the balance in efficient denitrification but avoid sulphate reduction and N2O formation in the woodchip bed and avoid harmful metal dissolution in anoxic sand infilter. Furthermore to find suitable operational parameters for the wintertime needs to be carefully studied.

Acknowledgements: The project was funded by The Ministry of Agriculture and Forestry, and Natural Resources Institute Finland.

**jouni.vielma@luke.fi*

Pikeperch in RAS - experiences and challenges

Marcus Stüeken[1]*, Tobias Rapp[1], Ralf Bochert[2]

[1]Mecklenburg-Vorpommern Research Centre for Agriculture and Fisheries, Hohen Wangelin, Germany; [2]Mecklenburg-Vorpommern Research Centre for Agriculture and Fisheries, Born a. Darß, Germany

Abstract

The pikeperch (*Sander lucioperca*) is a promising species for the diversification of production in recirculating aquaculture systems (RAS) and could prevent a local fishing pressure. It is highly valued by consumers, offers a steady sales market, achieves high market prices and as such may offer a sound investment for fish farmers in the future. Currently production costs of pikeperch in RAS are high, which requires the development of innovative procedural solutions to increase production volume and concurrently decrease costs per production unit.

In 2011, the Mecklenburg-Vorpommern Research Centre for Agriculture and Fisheries (Landesforschungsanstalt, LFA) initiated a research project on pikeperch aquaculture to study each aspect of pikeperch production in RAS on the vicinity of a meat production company in Hohen Wangelin. Based on previous cultivation trials in small-scale experimental facilities, the LFA decided to evaluate the potential of this species as an aquaculture candidate. The overall aim is the year-round supply of sufficient quantities of high-quality fingerlings. However, the supply of large quantities of vital fingerlings represents the highest matter of expense in production and still poses the main challenge.

As a recent result of the project, pikeperch fingerling production in RAS was significantly increased, achieved by optimization of the rearing environment, improvements in the hatchery and nursery protocols. The research facility hosts 14 specialized recirculating aquaculture systems with a total water volume of over 400 m³ and a production volume of 220 m³. Thus, some systems are used only for a few days to provide the fish with the best possible conditions.

Scientific studies are currently being carried out on various topics. 1) The artificial propagation of broodstock by simulating seasons in light- and temperature-controlled chilling rooms. Previous studies have shown that not only the temperature but also the light conditions during the reproductive cycle and during the growout in pikeperch play a major role and may also affect the quality of the gametes. 2) Testing of innovative first feeding and weaning protocols. By feeding the larvae a significantly higher survival could be ensured by the use of marine live feed as rotifers and different nauplii of copepods. 3) Improving the water treatment systems of RAS. This is crucial for compliance with given governmental thresholds and the saving of the resource water. Particularly elevated phosphorus levels demand additional water.

Overall, all production steps and results are evaluated with regard to their economic viability and are thus of relevance for producers. However, in order to preserve the natural resources, ecological sustainability is of central importance.

Acknowledgements: This research was funded by European Maritime and Fisheries Fund (EMFF) and by the Ministry of Agriculture and the Environment of the State of Mecklenburg-Vorpommern.

**m.stueeken@lfa.mvnet.de*

The challenges of raising yellowtail kingfish in marine RAS

Bram Rohaan*

Kingfish Zeeland, PM Kats, the Netherlands

Abstract
Kingfish Zeeland produces yellowtail kingfish in it's land-based facilities in the Netherlands. Current production capacity is 500t annually and expansion to 1000t will start in the end of the year. Kingfish Zeeland consists of two sites, with multiple broodstock units and two hatcheries, being able to provide fingerlings all-year round. The grow-out consists of 5 individual systems, where fish are grown from fingerling to harvest size fish up to 4,5kg.

Raising a pioneering species in a marine RAS has some challenges. Standard industry principles do not always apply in this respect. An overview of the challenges and successes are provided in the presentation.

**bramrohaan@gmail.com*

Nordic workshops on health and welfare of fish reared in RAS

Paul J. Midtlyng*

Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Oslo, Norway

Abstract

Since 2014, a project group consisting of fish health specialists from Finland, Denmark and Norway have organized two open scientific workshops specifically addressing health, diseases and animal welfare of fish reared in recirculation aquaculture systems (RAS). During the first meeting held in Vantaa, Finland early February 2015 with more than 70 attendants from Nordic, Baltic and North European countries could listen to 18 presentations including overviews of predominant health issues; case and field reports; on disease control and treatment; and on stocking densities and welfare monitoring. The presentations from the first workshop are still available from the Finnish Veterinary Laboratories' webpages:
http://aineisto.ruokavirasto.fi/evira20181231/www/en/about-evira/events/material-for-the-events/elintarvikkeet/3.-4.2.2015-workshop-on-fish-health-management-and-welfare-in-recirculating-aquaculture-systems-ras/index.html

A second workshop on the same topic was carried out in Oslo, Norway in November 2018 with some 140 registered participants. More than 25 scientific presentations and two posters were given, on new developments in fish health; infectious and non-infectious diseases; disease control and biosecurity; and fish health and well-being. Most of the presentations from the second workshop are available from http://marlife.org/program-2018-nordic-ras-workshop/

With our participation in the 5th Nordic RAS workshop in Berlin, signs are set for collaboration between the Nordic RAS Network and the Health and Welfare in RAS project group in preparation of a next international workshop comprehensively addressing both RAS technology, water environment, production, and the health and welfare of RAS inhabitant in the Oslo area, Norway during 2021.

Acknowledgement: Thanks to the Nordic Council of Ministers for Fisheries, Aquaculture, Agriculture, Food and Forestry for provision of generous financial support to the workshops.

*paul.midtlyng@nmbu.no

Knowledge holes - need to know or nice to know? Messages from the industry

Kjell Maroni*

The Norwegian Seafood Research Fund - FHF, Oslo, Norway

Abstract

"The Norwegian aquaculture industry is a world leading food industry, where salmon is its main product. The world leading position has been achieved through intensive investments in R&D from the beginning of the salmon industry about 40 years ago. R&D activities are conducted at a very high level, internally from industry companies themselves, from the Norwegian government, and from the Norwegian Seafood Research Fund – FHF…

FHF is a [Norwegian] state-owned limited company owned by the Ministry of Trade, industry and fisheries, and financed by the industry through a levy on exports of Norwegian Seafood at 0,3 %. FHFs goal is to create added value to the seafood industry through industry-based research and development (R&D)… In Aquaculture FHF adresses environmental documentation and impact, product quality, fish health and welfare, feed and feed resources as well as analyses of the formal framework for the industry." (https://www.fhf.no/fhf/about-fhf-english/)

**kjell.maroni@fhf.no*

Piscine orthoreovirus-3 (PRV-3), a new pathogen for farmed rainbow trout

Niccolò Vendramin[1]*, Juliane Sørensen[2], Argelia Cuenca[1], Maria Dahle[3], Anne Berit Olsen[4], Tine Iburg[1], Espen Rimstad[2], Niels Jørgen Olesen[1]

[1]DTU AQUA, Technical University of Denmark, Kgs. Lyngby, Copenhagen; [2]Department of Food Safety and Infection Biology, Norwegian University of Life Sciences, Oslo, Norway; [3]Norwegian Veterinary Institute, Oslo, Norway; [4]Norwegian Veterinary Institute, Bergen, Norway

Abstract

Piscine orthoreovirus – PRV have emerged as relevant pathogens for salmonid aquaculture worldwide. Three different subtypes of this viral species have so far been described:

- PRV-1 is the causative agent of heart and skeletal muscle inflammation (HSMI) in Atlantic salmon and is associated with jaundice syndrome in farmed Chinook salmon
- PRV-2 causes erythrocytic inclusion body syndrome (EIBS) in Coho salmon
- PRV-3 causes heart pathology resembling HSMI in rainbow trout

PRV-3 was firstly discovered in 2013 in Norway during disease outbreaks affecting farmed rainbow trout. The Norwegian PRV-3 isolate has been characterized by performing full genome sequencing and demonstrating causative relationship between the infection and the development of heart pathology in Rainbow trout.

An experimental infection study with purified virus demonstrated that PRV-3 infects rainbow trout and induces pathological heart lesions similar to Heart and Skeletal Muscle Inflammation (HSMI)

During 2017 the presence of PRV-3 was also reported in different countries in Europe. Interestingly, these viral isolates appear to be genetically distinct from the Norwegian isolate leading to proposition of two separate clades within PRV-3 viral type (PRV-3a and PRV-3b).

In Denmark the virus has been associated with severe disease outbreaks in recirculating aquaculture systems. Clinical signs are represented by reduced appetite followed by uncoordinated swimming behavior and increased mortality; necropsy findings include severe anemia and ascites. Such outbreaks are complex disease cases where different bacterial (including *Flavobacterium psychrophilum* and *Renibacterium salmoninarum*) and viral pathogens (IPNV) are present at the farms. Notably PRV-3 load increases in the target organs (heart, spleen) before the clinical disease appear, whereas the other pathogens are not detected in a systematic pattern.

In 2018 in cooperation with the Danish aquaculture industry, the presence of the virus has been mapped in the country, and a comparative study including the 2 PRV-3 subtypes conducted.

The preliminary results show suggests that PRV-3b is capable of faster replication than PRV-3a inducing higher innate inflammatory response and more severe heart pathology.

Results will be presented and discussed.

Acknowledgements: This research was funded by Henriksens Fond.

*niven@aqua.dtu.dk

Elevated CO_2 impacts growth and respiratory performance in yellowtail kingfish (*Seriola lalandi*)

Javed R Khan[1]*, Tony Pan[2], Dave McQueen[1], Steve Pether[1], Alvin N Setiawan[1], Neill A Herbert[2]

[1]National Institute of Water and Atmosphere, Northland Marine Research Centre, Ruakaka, New Zealand; [2]Institute of Marine Science, University of Auckland, Leigh, New Zealand

Abstract

Accumulation of dissolved-CO_2 is a central issue in intensive RAS. Long-term exposure of fish to high CO_2 leads to hypercapnia, diminished growth and increased production costs. However, CO_2 removal also incurs a substantial cost.

This research was undertaken to determine the effect of CO_2 exposure in yellowtail kingfish (*Seriola lalandi*) to inform cost/benefit analyses in yellowtail kingfish RAS design and management. Juveniles (~230 g) were reared for 54 days under the following average [CO_2] regimes; (1) 3.3 mg L^{-1}, pH 7.47, (2) 12.5 mg L^{-1}, pH 6.85, (3) 19.8 mg L^{-1}, pH 6.67, (4) 28.2 mg L^{-1}, pH 6.53, and (5) 39.6 mg L^{-1}, pH 6.38. Growth rates were highest in the two lowest [CO_2] treatments. Surprisingly, feed conversion efficiency was maintained up ~20 mg L^{-1} with a progressive increase in the higher [CO_2] treatments. Swim-flume respirometry revealed that standard metabolic rates were higher in kingfish reared at ~20 mg L^{-1} [CO_2] compared to ambient conditions, leading to reduced aerobic capacity.

Some operational data will also be presented which elucidates the accumulation rates of CO_2 in culture tanks at high densities in relation to feeding intensity, temperature and side-stream CO_2 reduction technologies.

**javed.khan@niwa.co.nz*

Investigating the use of peracetic acid to reduce saprolegniasis in Atlantic salmon RAS culture

Christopher Good[1*], John Davidson[1], Travis May[1], Curtis Crouse[1], Christine Lepine[1], Natalie Redman[1], Megan Murray[1], Steven Summerfelt[2], David Straus[3], Susan Harper[4], David Marancik[5], Timothy Welch[6], Brian Peterson[7], Lars-Flemming Pedersen[8], Vipaporn Phuntumart[9]

[1]The Conservation Fund's Freshwater Institute, Shepherdstown, WV, USA; [2] Superior Fresh, Hixton, WI, USA; [3]USDA-ARS, Stuttgart, AR, USA; [4]USDA-ARS, Beltsville, MD, USA; [5]St. George's University School of Veterinary Medicine, Grenada, West Indies; [6]USDA-ARS, Leetown, WV, USA; [7]USDA-ARS, Franklin, ME, USDA; [8]Danish Technical University, Hirtshals, Denmark; [9]Bowling Green State University, Bowling Green, OH, USA

Abstract

Despite numerous biosecurity benefits provided by land-based, closed-containment water recirculation aquaculture system (RAS) operations, opportunistic pathogens, such as *Saprolegnia* spp. oomycetes (causative agents of saprolegniasis) can still represent disease threats if environmental conditions are unfavorable, during vulnerable fish life-stages, or following stressful procedures. Saprolegniasis in general is associated with enormous losses in Atlantic salmon *Salmo salar* aquaculture, especially during the early rearing fry stage, smoltification, and following vaccination. We have investigated strategies to reduce saprolegniasis during each of these high-risk production cycle stages; however, at the time of abstract submission, only one study has been completed, while the remaining two are ongoing and will be presented at the workshop. To assess post-vaccination saprolegniasis, we applied daily peracetic acid (PAA) bath treatments at three dosages while evaluating the effects of these treatments on biofilter performance in replicated (12) experimental-scale RAS stocked with Atlantic salmon parr (200 fish per RAS, 94g mean weight) following intracoelomic injection vaccination. The PAA doses assessed were 0.2, 0.5, and 1.0 mg/L, and these were applied to each culture tank for a period of six weeks post-vaccination. Survival, waterborne *Saprolegnia* spp. colony counts, gill, spleen, and kidney histopathology, and biofiltration function (measured by total ammonia nitrogen (TAN) removal efficiency), were assessed. Visible skin lesions and fin erosion, hemorrhage, and observable *Saprolegnia* spp. infection were also evaluated. While no major post-vaccination saprolegniasis occurred in this study, survival was statistically ($p<0.05$) lower in control salmon, and visible saprolegniasis was significantly more prevalent in the control group. Despite less saprolegniasis being associated with PAA treatment, all treatment groups were associated with significantly lower fish weight by study's end. Biofilter TAN removal efficiency was not impacted by PAA administration at all dosages. Results suggest that low-dose PAA might be effective in reducing post-vaccination saprolegniasis while not significantly impacting RAS biofiltration. Further research, however, is necessary to confirm these findings, especially in commercial settings experiencing significant issues with saprolegniasis.

Acknowledgements: The post-vaccination research was funded through the US Department of Agriculture, Agriculture Research Service (USDA-ARS); the fry and smolt studies were funded through University of Wisconsin Sea Grant, part of the National Sea Grant College Program overseen by the National Oceanographic and Atmospheric Administration (NOAA).

cgood@conservationfund.org

An overview of the beneficial effect of continues ozonation in RAS

Aikaterini Spiliotopoulou[1]*, Richard Martin[1], Henrik-Rasmus Andersen[2]

[1]Water ApS, Farum, Denmark; [2]Department of Environmental Engineering, Technical University of Denmark, Kongens Lyngby, Denmark

Abstract

Recirculating aquaculture systems (RAS) have become increasingly important as fish are farmed in treated and reused water, achieving production continuity. The restricted use of make-up water, the high production intensity and feed loading deteriorate the water quality making the installation of water treatment technologies a necessity. Although ozonation has been applied for years in aquaculture, there is lack of knowledge regarding the reaction kinetics, the control of the dosage and side effects of excessive ozonation. The risk of losing fish or damaging the biofilters leads to a reluctance of the aquaculture managers to integrate ozone in RAS.

The present study aims to design a continues ozonated RAS with improved water quality by using fluorescence to continuously measure and control ozone in aqueous solution. The analysis of a few mL of water sample in the laboratory was sufficient to determine the ozone demand and the ozone lifetime of the system but also to predict the ozone dosage that was required for the pilot and/or full-scale RAS with remarkable proximity, achieving improved water quality without affecting the fish health.

The high sensitivity of fluorescent dissolved organic matter to ozone and its selectivity to specific fluorescent components, suggested that fluorescence could be used as an online sensor to control the organic matter in a RAS and determine indirectly the delivered ozone dosage in the system. This study attempts to clarify misinterpretations regarding ozonation and to offer new technological concepts to make its implementation safe, convincing the aquaculture managers to integrate ozone in their water treatment processes.

Acknowledgements: This research was funded by Innovationsfonden and Oxyguard international A/S. Authors are gratefull to DTU Aqua, DTU Environment for providing the facilities and Water ApS for equipment and technical assistance.

**ks@water.dk*

Production of lumpfish in RAS and FTS with distinct water treatments: Effects on fish survival, growth, gill health and microbial communities in rearing water and biofilm

Dahle, S. W[1]*., Nordøy, K[2]., Birkeland, M[3]., Bakke, I[3]., Attramadal, K.J.K[3]

[1]SINTEF Ocean, Trondheim; [2]Let Sea AS, Sandnessjøen; [3]NTNU Department of Biotechnology and Food Science, Trondheim. All Norway

Abstract

Lumpsucker in Norway is currently produced in traditional flow through systems (FTS). Hatcheries frequently show signs of bacterial infections, unstable microbial communities in the rearing water and varying mortality. RAS is proposed to create stable and healthy microbial environments, with less probabilities for blooming of opportunistic microbes. Studies have also shown that RAS increases the survival of marine fish. The aim of this study was to test the hypothesis that that rearing of lumpsucker juveniles in RAS will result in higher survival, growth and better fish health than FTS. We also predicted that lumpsucker juveniles reared in different set ups of RAS and traditional FTS will be exposed to distinct microbial environments.

An experiment with lumpfish was conducted at Ecomarine Seafarm AS in Norway, from 2 months post hatch to the transfer into sea cages. Five different systems, representing distinct water treatment regimens were conducted: 1) a RAS, 2) a RAS with additional mechanical filtration, 3) a RAS with filtration and UV, 5) a RAS with filtration, UV and ozone, 5) a FTS. Samples for characterization of the microbial communities from rearing water and biofilm were sampled throughout the experiment and characterized by 16S amplicon sequencing. In addition, the concentration of total bacteria, fraction of opportunistic bacteria, survival, growth, and gill health were measured during the experiment.

The microbial community composition of the rearing water in all the treatments were significantly different from each other, except the water microbiota from RAS and RAS Filtration, that were similar. We suggest that the disinfection strategy (none, UV, UV and ozone) significantly affected the water and biofilm communities. Further, the RAS without disinfection and with an additional filtration step resulted in more stable microbial environments for the cultured fish, compared to the FTS and the two RAS with disinfection. Also, the survival of larvae, growth and gill health was higher for fish reared in the different RAS compared to the FTS. This study shows that there is an apparent potential increasing the growth, health, and survival of lumpfish by designing the water treatment system for optimized microbial water quality.

Acknowledgements: This research was funded by RFF Nord, Norwegian research council, SINTEF and NTNU.

**Stine.w.dahle@sintef.no*

High resolution monitoring of sulphate-reducing and other priority bacteria in RAS

Roman Netzer[1]*, Deni Ribičić[1], Stine W. Dahle[1]

[1] SINTEF Ocean, Brattørkaia 17C, 7010 Trondheim, Norway

Abstract

In recent years, recirculating aquaculture system (RAS) technology has advanced rapidly in salmon smolt production and is increasingly applied in the sector. Even though designed for optimizing growth conditions, water quality in RAS is threatened by severe risks, often associated with the microbiota present at different locations in such systems. Besides the vital function of microorganisms for water cleaning and recirculation, such as nitrification, denitrification and degradation of organic material, microbes can also have a direct effect on the health of fish. While some bacteria can impact fish health positively (e.g. probiotic bacteria), pathogenic and opportunistic microbes represent an eminent threat, especially in closed systems, such as RAS. Also, non-pathogenic microbes, such as sulphate-reducing bacteria (SRB), can negatively affect fish health/welfare and robustness by converting sulfate to toxic hydrogen sulfide (H_2S) under anaerobic conditions. Sudden mass mortality is one of the major threats in salmon smolt RAS.

In the past years, an increasing number of such incidents has been reported and most cases have been associated with H_2S. Typical characteristics of such incidents include rapid and massive mortality, no obvious indications before the incident, lack of evidence for the real cause and no effective counteractions. Due to high sulfate concentrations, the risk of bacterial H_2S formation is high in systems operated with seawater. However, since mass mortality has also been associated with H_2S formation in fresh and brackish water systems, the problem cannot be exclusively reduced to sulfate concentrations in the water.

Even though H_2S has been a major concern in RAS for years and economic consequences are severe, reliable data is scarce, and information published is mainly based on speculations, opinions and practical experiences. Next generation sequencing technologies, such as 16S rRNA gene amplicon sequencing, are frequently employed to characterize microbial communities in environmental samples, including water, biofilm and sludge samples from RAS. Such methods provide detailed information on relative abundances of microbes present in a given environment. However, absolute concentrations of microbes of interest can typically not be determined.

At present, microbiological analyses used for quantification of microorganisms in RAS are mainly limited to low-coverage methods such as (semi)quantitative real-time PCR (qPCR), cell counting and agar plating. The advent of digital PCR (dPCR) technology has opened the door for absolute and ultra-sensitive quantification of microbes in environmental samples. We have employed dPCR for absolute quantification of total bacteria, SRB and other bacteria relevant for water quality in salmon RAS. Systematic quantitative monitoring of such priority microbes at relevant positions in RAS has the potential to significantly increase operational control and documentation and become a central element in early warning/decision support tools.

roman.netzer@sintef.no

Hydrogen sulfide production from fish organic waste

Carlos O. Letelier-Gordo[1]*, Sanni L. Aalto[1,2], Suvi Suurnäkki[2]

[1]DTU Aqua, Technical University of Denmark, Hirtshals, Denmark; [2]Department of Biological and Environmental Science, University of Jyväskylä, Finland

Abstract

The production of hydrogen sulfide (H_2S) has become a new challenge in marine land-based recirculating aquaculture systems (RAS). H_2S is a toxic gas that causes massive fish mortality even at low concentrations, and additional serious odour problems in the surroundings. It is a bacterial by-product originating from the degradation of organic matter in sulfur-rich waters e.g. marine waters with sulfate concentrations.

In order to develop methods to hinder H_2S production in marine land-based RAS, more information on the H_2S production conditions and microbiology is needed. This study followed the production of H_2S from rainbow trout (*Oncorhynchus mykiss*) organic waste under different salinities (0, 5, 10, 15, 25 and 35 ppt) in anaerobic mixed reactors, and examined the microbial community as well as abundance of sulfate reducing bacteria (SRB).

The results showed that H_2S formation increased with salinity, the maximal concentration being 23.1 ± 8.2 mg H_2S/L at 0 ppt and 153.9 ± 34.1 mg H_2S/L at 35 ppt. Similarly, the H_2S production rates increased from 5.6 ± 0.2 to 26.4 ± 12.7 mg of H_2S produced per day with increasing salinity. The H_2S concentrations normalized with total chemical oxygen demand (TCOD) ranged between 0.76 - 7.21 mg H_2S per gram of TCOD, being significantly lower in 0 ppt treatment than in higher salinities, where no differences were found.

The overall microbial community, measured in experiment 2, changed gradually in time and between salinities, phyla Fusobacteria and Bacteroidetes being more abundant in 35 ppt reactors than in other reactors. The common SRB were found only in 0 and 5 ppt reactors, while in 10 ppt and 35 ppt reactors, H_2S production was driven by novel currently unidentified SRB groups.

The presented study is an important finding contributing to fill up essential knowledge gaps, providing a reliable quantifiable method for H_2S measurements and new information for understanding H_2S production in aquaculture systems.

Acknowledgments: This work originated from the BONUS call 2015: Blue Baltic – BONUS CLEANAQ project, supported by BONUS (Art 185) and funded jointly by the EU and Innovation Fund Denmark.

**colg@aqua.dtu.dk*

The effect of different water sources on the potential H_2S-formation within RAS

Paula Rojas-Tirado[1]*, Åse Åtland[1], Carlos Letelier-Gordo[2]

[1]NIVA, Norwegian Institute for Water Research, Section for Aquaculture, Bergen, Norway; [2]DTU Aqua, Technical University of Denmark, Hirtshals, Denmark

Abstract

In the last years, several of the serious incidents involving acute fish mortalities in recirculating aquaculture systems (RAS) for Atlantic salmon (*Salmo salar*), have been caused by hydrogen sulphide (H_2S). These incidents have mainly occurred in seawater systems, e.g. post-smolt production. H_2S is formed by sulphate-reducing bacteria which uses sulphate (SO_4^{2-}) and organic material under anaerobic conditions. Seawater contains 1000 times more SO_4^{2-} than freshwater, increasing the potential risk for H_2S production. However, using seawater is pivotal to avoid desmoltification and preparing salmon for seawater transfer. The project where this preliminary study is from, propose removing sulphate from seawater through membrane filtration as a measure for reducing fish mortalities caused by H_2S.

The aim of this preliminary study was to understand what microbial environments in RAS have the highest potential risk for H_2S-formation and to gain a better understanding of the dynamic between organic material and sulphate concentration for H_2S formation in RAS-water.

Three main environmental sources where H2S could potentially form in a commercial RAS were selected: sludge, biofilter elements and RAS-water. A small-scale batch experiment was conducted where each of these three potential sources were exposed to seawater and brackish RAS-water. The H_2S kinetics and production rate was measured for each test. The organic material was also measured in form of COD (chemical oxygen demand) and organic carbons as fatty acid. Anions such as NO^{3-} and SO_4^{2-} and other water quality parameters were assessed.

The results of this experiment are still under development and analysis. Therefore, the results will be presented at the 5th NordicRAS workshop.

Acknowledgements: This research was funded by Innovasjonsprosjekt i næringslivet-HAVBRUK2 program through the project “Sulphate removal by membrane filtration technology to reduce the risk of fish death due to H_2S formation in recirculating aquaculture systems (RAS) – Aquasulfat” (Project number: 296545).

**paula.rojas@niva.no*

Trends and distribution of geosmin across RAS compartments: control and management of fluctuations and outbursts of geosmin

Raju Podduturi[1]*, Martin Vestergaard[2], Thue Holm[3], Niels O. G. Jørgensen[1]

[1]Microbial Ecology and Biotechnology, Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg, Denmark; [2]Aquapri Denmark, Vejen, Denmark; [3]Atlantic Sapphire, Hvide Sande, Denmark

Abstract

The presence of bacterial metabolites in aquaculture production facilities and in fish products is a globally occurring issue. Geosmin is one of the major off-flavoring compounds in cultured fish which taints the fish by imparting earthy flavor. The knowledge on dynamics of geosmin concentrations in RAS is very limited. Since cultivation of fish in RAS is a continuous process with constant water treatment and system maintenance, any unintentional operational procedure or shift in microbial populations may cause episodes of high geosmin concentrations. Thus, knowledge on geosmin dynamics, relative to RAS operations, might help preventing such episodes.

Two full-scale commercial RAS systems, one fresh water RAS rearing pikeperch (Stizostedion lucioperca) and one salt water RAS rearing Atlantic salmon (Salmo salar) were sampled in this study to investigate contribution of various RAS compartments and daily operations on geosmin dynamics. The obtained results showed only a minor increase in geosmin during recirculation of water through the production tanks. Geosmin contribution from the biofilters varied between the systems. In fresh water RAS, water passage through the biofilters did typically not affect the geosmin concentration, where as in saltwater RAS, geosmin levels in outlets of biofilters varied significantly, apparently depending on the age of biofilter. The denitrification unit caused a 3.5- to 5-fold increase in the geosmin content, while the geosmin level decreased during passage of the trickling filters.

In both fresh- and salt water RAS, cleaning of biofilters and denitrification units caused a temporarily outburst of geosmin up to 100-200 ng/L (biofilters) and 210 ng/L (denitrification unit), as compared to typical levels of 10 to 30 ng/L in the water, suggesting cleaning of filters to be one of the major hotspots for increased geosmin levels. It was found that increased cleaning frequency with additional back washes and bio-solid removal while cleaning biofilter seemed to limit the contribution of biofilter cleaning to the systems geosmin.

**podduturi@plen.ku.dk*

Availability of biodegradable polymers as a biofilter in freshwater recirculating aquaculture systems for rainbow trout *Oncorhynchus mykiss*

Nobukazu Satoh[1]*, Yoshitomo Katsumata[1], Mahito Miyamoto[1], Ryohei Yasutomi[1], Mizue Murooka[1], Mari Hara Yasuda[2], Tomoki Kawagish[3]

[1]Salmon and Freshwater Fisheries Research Institute, Hokkaido Research Organization, Hokkaido, Japan; [2]Planning Div., Environment and Living Solutions Business Domain, Mitsubishi Chemical Corporation, Tokyo Japan; [3]Tsurumi Research Center, Mitsubishi Chemical Corporation, Japan

Abstract

Nitrate nitrogen (NO_3-N) concentrations causing physiological abnormalities in rainbow trout (*Oncorhynchus mykiss*) have been clarified until present. In recirculating aquaculture systems (RAS), nitrate removal from rearing water is a big challenge.

We conducted two preliminary experiments to evaluate the availability of biodegradable polymers (BP) as a biofilter within RAS for rainbow trout. In experiment 1, we carried out a nitrate removal experiment using two RAS ($2m^3$ tank) rearing rainbow trout (initial mean body weight 203.5 g) at a density of $17kg/m^3$ during 313 days, and found it worked well under low suitable temperature for trout culture. However, rainbow trout in this RAS showed low growth performances and survival rates, and the cause was insufficient physical filtration in RAS.

In experiment 2 using two RAS ($0.25\ m^3$ tank) with improved physical filtration, we investigated the availability of BP as a biofilter for nitrate removal during 70 days. Each RAS was stocked with rainbow trout (initial mean body weight 127.7g) at a density of $16kg/m^3$. The first RAS (RAS 1) was operated as a control without nitrate removal unit. The second RAS (RAS 2) was operated with a biofilter unit containing BP. In both RAS 1 and RAS 2, we used an intermittent biofilter system containing biological filter media derived from sea urchin skeletons for nitrification.

Mean NO_3-N concentrations (ppm) for the duration of RAS 1 and RAS 2 treatments were 139.5 and 35.3, respectively. In both treatments, there were no significant differences in growth and hematological characteristics of fishes and survival rates were also 100%. Final density values (kg/m^3) for RAS 1 and RAS 2 treatments were 23.4 and 24.4, respectively.

These results show that BP is effective for nitrate removal in rainbow trout fresh water RAS.

In separated RAS experiment of brackish water, we found a tricking filter unit containing BP worked well to remove nitrate. This result indicates that we could apply this aerobic nitrate removal system for post-smolt salmon culture in brackish RAS.

**sato-nobukazu@hro.or.jp*

Microbiology of woodchip bioreactors treating RAS effluents

Sanni Aalto[1,2]*, Mathis von Ahnen[2], Suvi Suurnäkki[1], Per Bovbjerg Pedersen[2], Marja Tiirola[1]

[1]Department of Biological and Environmental Science, University of Jyväskylä, Finland; [2]DTU Aqua, Technical University of Denmark, Hirtshals, Denmark

Abstract

Woodchip bioreactors offer low-cost nitrate removal applications for treating recirculating aquaculture system (RAS) effluents. Therein, nitrate removal is based on active microbial biofilms, to which woodchips provide growth surface as well as an additional carbon source. However, the microbiology of woodchip bioreactors is still poorly known. In addition, the interactions between bioreactor conditions, microbial community and nitrate removal routes have not been described. Here, we combined stable isotope approach to molecular microbiology tools for studying the overall microbial community, the nitrate removal dynamics, and the abundance and community composition of microbes involved in nitrogen transformation processes in three full-scale woodchip bioreactors treating RAS effluents.

We found that water and biofilm of the woodchip bioreactors were hotspots of bacteria and fungi. Complete denitrification leading to N_2 production was the main nitrate removal pathway, but N_2O production was substantial in bioreactors with high nitrate concentrations and low amounts of bioavailable carbon, and DNRA (dissimilatory nitrate reduction to ammonium) rates increased in the nitrate-limited bioreactors. The abundance of denitrifying genes was similar among the three bioreactors, suggesting that the nitrate removal dynamics were controlled by the load of nitrate and bioavailable carbon rather than by the lack of genetic potential. However, the abundance of DNRA and sulfate-reducing microbes increased under nitrate limited conditions, suggesting that too long hydraulic retention times in relation to nitrate loading can increase H_2S production and reduce nitrogen removal.

The overall microbial communities, and especially denitrifying communities, were similar among the bioreactors. Denitrification was driven by certain core proteobacterial groups, while DNRA was carried out by groups belonging to Bacteroidetes. Nitrate removal was linked to the diversity of the denitrifying community, wherein high diversity and species richness of *nir*S-carrying microbial community supported complete denitrification with N_2 production.

Altogether, our results show that while woodchip bioreactors host a high genetic potential for nitrate removal, the rate and route of nitrate removal depends on the bioreactor design, retention time and amount of bioavailable carbon.

Acknowledgements: This research was funded by BONUS (Art 185), funded jointly by the EU and national funding institutions of Finland (Academy of Finland), Sweden (Vinnova) and Denmark (Innovation Fund Denmark IFD).

**sanni.lh.aalto@jyu.fi*

Addressing unsolved questions regarding denitrifying woodchip bioreactor operation at RAS

Mathis von Ahnen[1]*, Per Bovbjerg Pedersen[1]

[1]DTU Aqua, Technical University of Denmark, Hirtshals, Denmark

Abstract

The feasibility of using full-scale denitrifying woodchip bioreactors as a technologically simple and cost-effective method to remove nitrate from RAS effluents has been demonstrated at commercial RAS in Denmark, and new woodchip bioreactors are being established. However, more knowledge on how best to start up woodchip bioreactors is needed to avoid that initial leakage of potential hazardous compounds reach receiving water bodies. In addition, little is known about the capacity of woodchip bioreactors for removing disinfectants that are frequently applied in RAS.

In this presentation, results from on-going laboratory studies on a new potential technical solution on how to reduce the outflow of dissolved compounds from woodchip bioreactors during start-up will be presented. Furthermore, initial results from an ongoing trial investigating the removal of formalin and peracetic acid in woodchip bioreactors and potential effects on nitrate removal will be shown.

Acknowledgements: The research is funded by the Ministry of Food, Agriculture and Fisheries of Denmark and by the European Union through the European Maritime Fisheries Fund (EMFF).

**mvah@aqua.dtu.dk*

Nordic Council of Ministers

BioMar

5th NordicRAS Workshop on Recirculating Aquaculture Systems

Berlin, Germany, 7-8 October 2019

Day 1: Monday 7 October 2019

08^{00}-09^{30}	Registration
09^{30}-09^{45}	Opening session
09^{45}-11^{00}	Session I: RAS around the world
11^{00}-11^{30}	Coffee break
11^{30}-13^{15}	Session II: It all starts with feed – particles and how to remove them
13^{15}-14^{15}	Lunch
14^{15}-16^{00}	Session III: Microbial water quality in RAS
16^{00}-16^{30}	Coffee break
16^{30}-18^{00}	Session IV: RAS operations – experiences & challenges
19^{00}	Workshop dinner

Day 2: Tuesday 8 October 2019

08^{00}-08^{30}	Registration
08^{30}-10^{15}	Session V: Fish health & water quality in RAS
10^{15}-10^{45}	Coffee break
10^{45}-12^{30}	Session VI: Harmful and helpful microbial processes in RAS
-	Goodbye and see you next time

NordicRAS

DTU