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Towards biocide-free recirculating aquaculture systems

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1. PREFACE

This report was produced by the project '*Towards biocide-free recirculating aquaculture systems*' (TOBIFREE) that received funding from the pesticide research program of the Danish Environmental Agency in 2018-2021. The project was conducted in the Technical University of Denmark, National Institute for Aquatic Resources (DTU Aqua), at the sections of Aquaculture and Oceans and Arctic.

Several students contributed substantially for the gathering of the material as master students in the project, through special project courses or as student helps. The master thesis of Stephan Smith focused on the biofilter communities, the master thesis Leila Jafari on foam fractionation to improve water quality, the special course of Kathrine Tardum Franzén Storm on feeding and toxin accumulation of *Daphnia magna* and the special course of Stanley Gorzelnik on the potential of zooplankton to remove microalgae in small-scale recirculating systems. Linda Latuta and Ghebrehiwet Yacob Tesfa worked as student helpers in the project. Besides these students, we would like to thank Jack Melby, as well as fish technician Ole M. Larsen, Rasmus F. Nielsen, Jens H. Nedergård for the technical help during the project as well as valuable analytical support from laboratory technicians Ulla Sproegel and Brian Møller.

Finally, we would like to thank the aquaculture farmers and other professionals who helped by sending samples and by answering the survey.

2. LIST OF ABBREVIATIONS

β value – an deducted value describing the particle size distribution of microparticles

BOD_{5_Total} – Total Biological oxygen demand (5-days) from untreated samples

BOD_{5_Diss} – Dissolved Biological oxygen demand (5-days) from 0.22 μm filtered samples

BOD_{5_Part} – Particulate Biological oxygen demand (5-days) – as BOD_{5_Total} - BOD_{5_Diss}

COD_{Total} Total Chemical oxygen demand from untreated sample

COD_{Dissolved} Dissolved Chemical oxygen demand from 0.22 μm filtered samples

COD_{Part} Particulate Chemical oxygen demand as COD_{Total} - COD_{Diss}

Chl – Chlorophyll-a

ELISA - Enzyme–linked immunosorbent assay

FF - Foam fractionation

NTU- nephelometric turbidity unit

Ppm – part per million

PN - Total particle number

PSA - total particle surface area

PV - total particle volume

RAS - Recirculating aquaculture system

SSA - Specific Surface Area

TCOD – Total chemical oxygen demand

TSS – Total suspended solids

UVT – Ultra violet transmission at 254 nm

3. ABSTRACT

Recirculating aquaculture systems (RAS) have been promoted as a sustainable supplement to net pen aquaculture and land-based flow-through systems, and RAS is currently a commonly used production concept. RAS have numerous environmental assets such as decreased water consumption, but there are challenges related to water quality control and use of biocides in some systems. As the retention time and degree of reuse of water increases, the nutrients and organic matter accumulate causing favorable conditions for micro-organism growth, which can result in decreasing water quality including blooms of harmful micro-organisms. Treatment of these blooms includes application of disinfectants such as formalin, hydrogen peroxide and peracetic acid.

Recent new knowledge on the microbial dynamic and water quality in RAS has enabled development of alternative treatment methods, such as membrane filtration, UV, and ozonation, and preliminary studies have identified the potential of biological control of micro-organisms by their naturally-occurring zooplankton predators. However, these methods are not optimized and costly (physical control) or not tested in large-scale (biological control), which hampers their application in aquaculture systems. Further, the sources, dynamics and environmental control of the development of micro-organism blooms are still not understood. For instance, it is conceivable that biofilter could function as a 'ticking bomb' and that imbalances in the biofilter communities could be the source of micro-organism blooms in treatment tanks.

The main aim of our project 'Towards biocide-free recirculating aquaculture systems' (TOBIFREE) was to provide new knowledge of the causes and treatments of microalgal blooms in RAS systems that could result in reduction of the use of biocides in aquaculture. Specifically we wanted to investigate 1) the biofilter communities and whether they might act as a source for micro-organism blooms in growing tanks, 2) the potential alternatives to reduce the use of biocides, namely biological control by zooplankton and physical control by foam fractionation and ozone and 3) the barriers that aquaculture industry might have for the use of new treatment methods.

Biofilters harbored a rich community of protozoans and invertebrates such as copepods, ostracods, nematodes, polychaetes, rotifers and diverse eggs, and appeared to function as small ecosystems with active reproduction and predator-prey interactions and high turnover times. Dominating groups or species differed between the facilities, likely depending on salinity or light conditions, but were typically similar in the different systems at the same facility. Also, abundances of most organisms did not seem to change due to maintenance cycle, suggesting that the organisms resisted backwashing and remained in the system. Experiments investigating the interacting effects of propagule size, nutrient concentrations and the presence of a zooplankton (ostracod) suggested that ostracods that are naturally present on biofilters can control the abundances of microalgae, even at high nutrient concentrations. Similarly, diverse cladocerans had high feeding rates on microalgae, and particularly individuals that were collected from lakes with cyanobacteria blooms were able to feed on toxic cyanobacteria *Microcystis aeruginosa* at high rates.

Also, physical treatment methods were effective. Foam fractionation was a simple and effective water treatment technique to remove microparticles from freshwater RAS, and FF in combination with hydrogen peroxide and addition of salt led to significant reduction of both bacteria and turbidity. Pilot scale RAS trials documented beneficial properties of FF in terms of removal of microparticles, reduction of bacterial load, reduction of biodegradable organic matter and improvement of water clarity. FF combined with ozone led to an immediate and persistent improvement of water quality measured as bacterial load and microparticle concentrations. Both physical and biological treatment methods seem thus to be promising alternatives

to chemical water treatment. Whereas biological treatments are still relatively far from application, physical treatment methods could become a viable option for freshwater RAS in near future.

4. INTRODUCTION

4.1 Recirculating aquaculture systems and biocides

Production of fish in aquaculture systems accounts for the fastest growing food sector globally, exceeding the supply of wild-caught fish (FAO, 2020). Recent development in aquaculture industry includes rearing systems that reuse the water and allow for better control of production conditions and environmental impact (Heldbo Birke-land 2017; Xiao et al. 2019). These recirculating aquaculture systems (RAS) have been promoted as a sustainable supplement to net pen aquaculture and land-based flow-through systems, and RAS is currently a commonly used production concept, including land-based grow-out systems for Atlantic salmon, pike perch, and kingfish (Dalsgaard 2014).

RAS have numerous environmental assets such as decreased water consumption, but there are challenges related to water quality control and use of biocides in the systems. As the retention time and degree of reuse of water increases, the nutrients and organic matter accumulate causing favorable conditions for micro-organism growth, which can cause impairment of the production either due to their high abundance and consequently decreased water quality, or due to blooms of directly harmful organisms. For instance, in 2012, blooms of two heterotrophic dinoflagellates *Pfiesteria* sp. and *Luciella* sp. caused high fish mortality in two unrelated land-based RAS (Moestup et al. 2014). Particular challenge regarding the bacteria is caused by the fact that a substantial fraction of bacteria in RAS grow on surfaces as biofilm – some are difficult to control (heterotrophic bio-fouling) while others require much attention (nitrifying bacteria in biofilters).

The amount of water that is used for each kilogram of produced fish dictates the treatment requirements to obtain and ensure stable and optimal water quality (Fig. 4.1). Water disinfection is required at a certain recirculation intensity and in many RAS chemical disinfection is the common choice. Particularly in the model trout farms which now account for approximately half of the Danish trout production, ongoing water disinfection is required to maintain and / or control acceptable water quality. Water treatment includes application of disinfectants directly to the water, among which formalin (containing the biocidal agent formaldehyde and methanol) is used in considerable amounts. Formalin use has been reported to be > 250 000 L year⁻¹ in 2015, a quantity exceeding previous year, and most likely not to be reduced considering the frequent use in model trout farms. The use of other chemical disinfectants, such as hydrogen peroxide and peracetic acid, is similarly increasing.

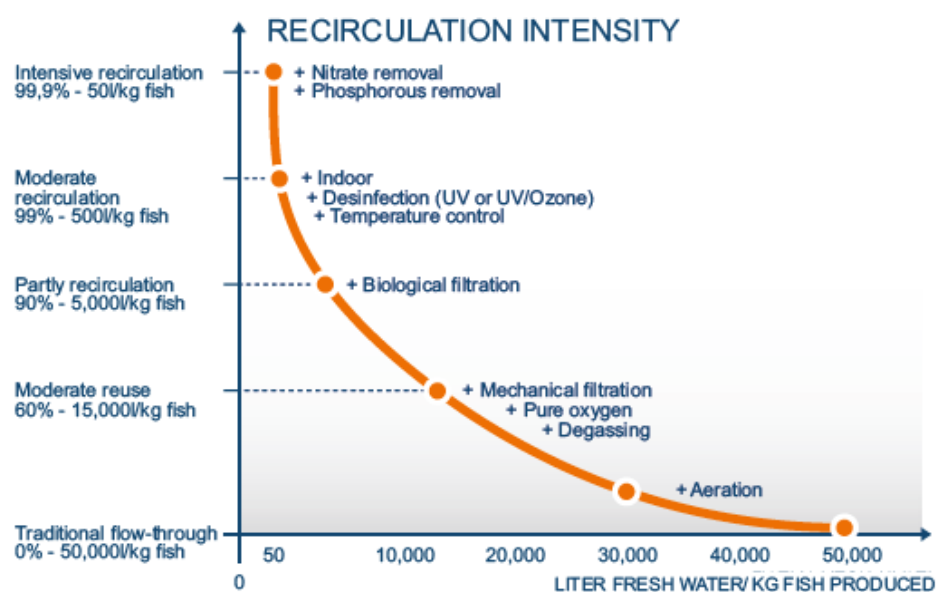


Fig. 4.1. Water quality issues and treatment options related to recirculation intensity. Aquaculture biocides are commonly used to improve microbial water quality by eliminating parasites and reducing bacterial loads in the water (modified from Heldbo & Birkeland. 2017).

4.2 Alternative water treatment methods

Until recently, water quality in RAS has mainly been assessed by traditional chemical measures. However, new methods assessing microbial abundance, activity, and community composition have provided new knowledge on the microbial dynamic and water quality (Dalsgaard et al. 2017; Gregersen et al. 2019; Pedersen et al. 2017; Pedersen et al. 2019), which has enabled development of alternative treatment methods (Table 4.1, Fig. 4.2). Membrane filtration, UV, and ozonation have all shown promising results in controlling microbial water quality, however, with substantial costs.

Also, the potential of biological control of micro-organisms in RAS by using their naturally occurring zooplankton predators has been tested (Pinyol Gallemi 2016). In aquatic environments, zooplankton feed on small primary producers (phytoplankton) and diverse micro-organisms, and are in turn food for larval fish. Zooplankton as a group includes a myriad of species, some of which (namely copepods) are considered to be the most abundant multicellular organisms in the world (Mauchline 1998). Zooplankton have diverse life-history strategies and feeding modes: Whereas some species feed on everything that is at a suitable size range (i.e., 10-20 times smaller than themselves; Hansen et al. 1994), some species are able to select for the most nutritious food particles (Kjørboe 2011). Zooplankton feeding and growth rates are relatively high, with temperature and species-specific generation times that range from days to weeks (Huntley & Lopez 1992) and feeding rates that allow them to ingest their own weight per day (Saiz & Calbet 2011). The high diversity, high abundance and potentially high ecophysiological rates make zooplankton good candidates for biological control. Also, some species are already reared in mass cultures, with the aim of providing live feed for cultured fish (Hansen 2017).

Many zooplankton species are able to feed on toxic algae, and small-scale experiments have demonstrated the ability of the water flea *Daphnia magna* and cyclopoid copepods originating from a bio-filter in a RAS system to feed on RAS-relevant toxic algae with high clearance rates (Fig. 4.3). In these experiments both tested zooplankton species fed on the three types of toxic microalgae – prymnesiophyte *Prymnesium parvum*, dinoflagellate *Pfiesteria* sp. and cyanobacteria *Microcystis aeruginosa* – at rates that were similar to the feeding rates on a non-toxic control algae, and the presence of zooplankton consequently induced a negative growth rate of the algae. Biological control could be particularly beneficial to treat micro-organisms that form resistant resting stages. For instance, peracetic acid in concentrations used in aquaculture merely triggers cyst formation in the toxic dinoflagellate *Pfiesteria* sp., with cysts germinating once the peracetic acid has degraded (Pinyol Gallemi et al. 2018). Biological control could also be preferred if the micro-organisms develop resistance against biocides.

TABLE 4.1. Overview of aquaculture related studies focusing on microbial water quality using different approaches to enhance, reduce, inactivate or eliminate bacteria from the water phase.

Treatment	Results	Ref
UV and particle filter	Improved microbial water quality, reduced bacterial activity	Gregersen et al. 2020; Huyben et al. 2020
Change in levels of feeding intensity	Migration of particles from the biofilter to water with ceased feeding	Rojas-Tirado et al. 2018
Ozone and foam fractionation	Reduced bacterial load and activity in the water phase	Figueiras et al. 2020 Gregersen et al. (in prep)
Acetate addition	No apparent effect on microbial water quality	Rojas-Tirado et al. 2019
Type of filter	Source/sink dynamics; liberation of particulate OM from moving bed biofilters	Fernandes et al. 2017 Pulkkinen et al. 2019
Disinfection and disturbance	Shift from slow growing to opportunistic bacteria	Blancheton et al. 2013
UV and ozonation of sea-water	Demonstration of regrowth potential after disinfection of ballast water	Hess-Erga et al. 2012

Mode of peracetic acid application	Continuous low dose of peracetic acid promotes biofilm growth	Liu et al. 2017; 2018
Sand filtration	Reduced biofilm formation	De Oliveira et al. 2019
Membrane filtration	Improved microbial water quality / reduced bacterial load	Wold et al. 2014




RAS conditions	Problems and consequences	Treatment options
<ul style="list-style-type: none"> - Input of feed (> 1000 kg/d) - Continuous excretion from fish (dissolved and particulate matter) - Long/prolonged retention time - Insufficient solids removal - Occasional feed loss 	<p>Accumulation of dissolved substances & microparticles</p> <p>Fluctuations and instability Deteriorated water quality, Bacterial growth</p> <p>Biofouling and anaerobic zones</p> <p>Reduced fish performance and increased mortality</p> <p>Need of using biocides</p>	<p>Chemical: Biocides, disinfectants, formalin, peracetic acid, hydrogen peroxide</p> <p>Mechanical: Drumfilter, fixed bed biofilters/contact filter, foam fractionator, membrane filter swirl separator</p> <p>Technical: Ozone, UV irradiation, ultrasound, electrochemical oxidation</p> <p>Biological: Grazers, daphnia, copepods pre- & probiotics, phages, bacterial inoculum</p>  

FIGURE. 4.2. Example of conditions in RAS, their potential problems and consequences for the water quality and the current treatment options.

4.3 Biofilter as a ‘ticking bomb’

Biofilter units are complex, and empirical evidence suggest that they might foster a rich community of small heterotrophs, zooplankton and benthic invertebrates Smith, 2019. Although no systematic studies exists on the biofilter communities, we have observed that biofilters can be brought out a balance in different ways. For example, change of rearing conditions (salinity change, altered feed allocation, organic matter pollution), management practices (backwashing, hydraulic changes) and use of biocides in the water phase passing the biofilter (from habituation to inactivation) can change the biofilter communities and possibly alter the interactions between the trophic levels (Rojas-Tirado et al., 2018). For instance, blooms of micro-organisms in RAS systems (i.e. Moestrup et al., 2014) could originate or be liberated from the biofilter, in case of unfavorable conditions (i.e. lack of carbon input and starvation) and natural predators such as benthic invertebrates or zooplankton organisms are disturbed and the harmful organisms are released from the predation pressure. Thus, we expect that a biofilter unit can potentially be considered to function as a “ticking bomb”, by trapping large amounts of organic matter, and potentially high densities/abundances of micro-organisms, in the biofilter that potentially can enter the water phase and reach the fish. This could cause sudden changes in system water quality and potentially acute or longer lasting detrimental effects for fish, typically oriented towards gill and skin. These types of sudden catastrophic events typically require water treatment with large amounts of biocides and can lead to mortality events or decreased fish performance, with the consequent economic impact on the farm. Understanding the biofilter communities and their role in maintaining the water quality could therefore be the key for early detection and reduction of the problems.

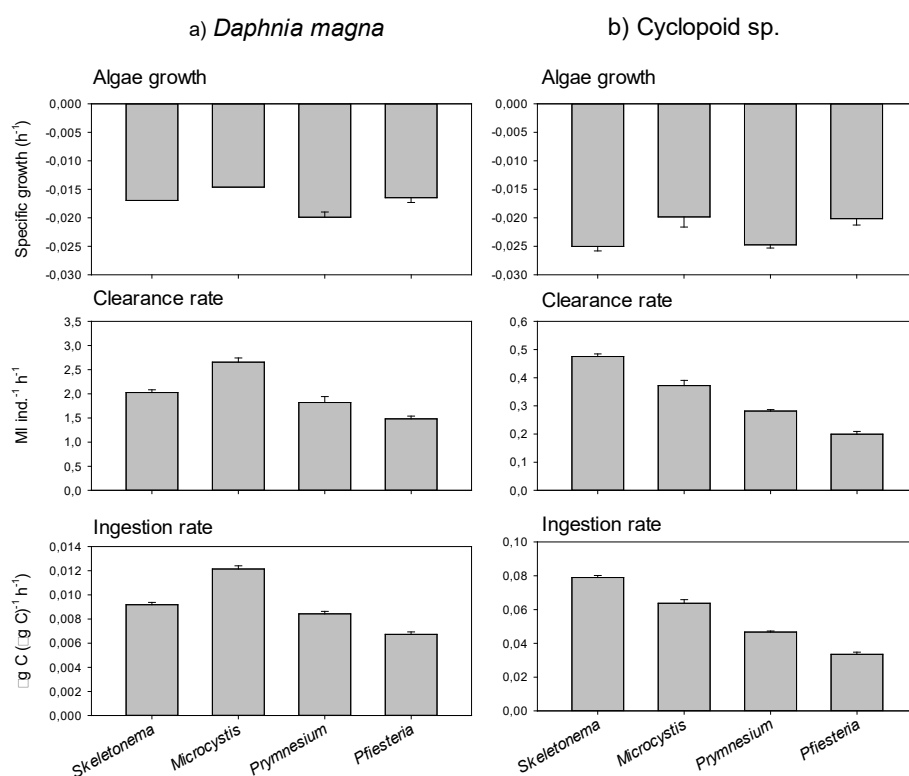


FIGURE 4.3. Algae growth rate (doubling time h⁻¹), and clearance (ml ind.⁻¹ h⁻¹) and weight-specific ingestion rates (μg C (μg C)⁻¹ d⁻¹) of a) *Daphnia magna* and b) unidentified cyclopoid species on diverse toxic algae (Pinyol Gallemi 2016).

4.4 Promotion of new technology

The global development and adoption of technology in the food sector is continuously ranked among the key instruments to ensure continued industrial advancements with benefits for both “people, profit and planet”, and are therefore heavily supported by major public funding institutions such as the European Union’s Horizon Programme (European Commission 2019). Publicly funded research and development (R&D) projects related to technology development and adoption come in many shapes depending on the scope and potential industries involved. In Danish aquaculture such projects have historically had a particular focus on advancing the ability to produce fish in freshwater ponds and later RAS. This has resulted in multiple projects focusing on water treatment, e.g., reduction of emissions of nutrients through discharge water, removal of pathogens, treatment of disease, or removal of unwanted bacterial metabolites. A recent example is the adoption of woodchip-based denitrification bioreactors in commercial model trout farms. Like similar projects also in other sectors (e.g., agriculture) these solutions have been heavily supported by continued R&D efforts by public universities, which have expanded the understanding of the “system” and thus lowered some of the potential barriers related to adopting the denitrification approach at farm level (e.g. von Ahnen et al. 2016; 2019).

Kumar et al. (2018) recently reviewed the evidence-base for a diversity of factors driving technology adoption. In this study, the technology adoption process in aquaculture were considered similar to that of other industries, and could be seen as a timeline consisting of the following phases: 1) awareness, 2) interest, 3) evaluation, 4) trial and 5) adoption/rejection. The potential barriers for aquaculture farmers to adopt new technology cover both economic, political, social and policy issues (App. Table 1). From an R&D point of view, these categories therefore need explicit attention if R&D projects are to become successful in facilitating transfer of knowledge and technology.

The technology uptake relies both on the perceived usefulness of the technology and on the perceived risk (Im et al. 2007). The European Commission has recognized that there is a “valley of death” (e.g. EC 2009) in order for new methods or technologies developed in e.g., universities to become available as new products or services. This is due to the risk perceived by companies that would need to invest in developing the technology, so that the willingness of adoption of a new method or technology by aquaculture farmers may be impacted by

their perceived risk of implementing it. Nevertheless, in a Danish context little is known about the perceived and actual risks as well as specific barriers, which could reduce the willingness of aquaculture farmers to adopt new technology.

4.5 Objectives

The main aim of our project '*Towards biocide-free recirculating aquaculture systems*' (TOBIFREE) was to provide new knowledge of the causes and treatments of microalgal blooms in RAS systems that could result in reduction of the use of biocides in aquaculture. Specifically we wanted to:

- Investigate the biofilter communities and whether they might act as a source for micro-organism blooms in growing tanks
- Investigate the potential alternatives to reduce the use of biocides, namely biological control by zooplankton and physical control by ozone
- Investigate the barriers that aquaculture industry might have for the use of new treatment methods

Below we cover the methods and results addressing these three main objectives, and outline the future research needs.

5. MATERIALS AND METHODS

5.1 Biofilter communities and their metabolic activity

Facilities and sample collection: Biofilter communities were sampled in four facilities (Table 5.1.1). Facility 1, Gamst Aquakultur (<https://www.aquapri.dk/>), is a modern commercial RAS, designed to produce 500 tons pike perch per year, with both brood stock facility and grow out tanks for several life stages. The samples were taken in the system C that had a total volume of 2500 m³, consisted of mechanical filters, UV-treatment, biofilters and trickling filters, and was used for growing out the fish in later life stages. The four biofilters in system C (C1-4) were models consisting of moving bed and fixed bed, with volumes of 100 m³, water flows of 800 m³ h⁻¹ and a water supply from below the filter. The biofilters were typically backwashed with a frequency of once to twice per month. The backwash was done by pushing compressed air from below the filter to the surface for ca. 15 min., after closing inlet and outlet of the biofilter units. The bubbling air made the water and filter media move around rapidly which released biofilm and biomass from the filter media. The water in the biofilter was then drained out and led to the sewer, the inlet and outlet valves were opened, the biofilter refilled with water and was again active. The samples from this facility were obtained weekly for a period of one month in April-May 2019, aiming to sample three filter systems (C1, 2 and 4) directly after the backwash, and ca. 1, 2 and 3 weeks after the backwash. The samples comprised of 1) water samples taken upstream and downstream from the biofilter (Fig. 5.1.1), 2) samples of three types of filter media (Fig. 5.1.1) and 3) samples from backwash water that contained both water and media.

Facility 2, Løjstrup Dambrug, is a freshwater outdoor facility growing trout (Table 5.1.1). The facility has several growing systems with mechanical and biofilters that are backwashed at regular intervals. The samples were taken from two systems with moving bed biofilters before, during and after backwash, in November 2018. The samples consisted of water and filter media samples before the backwash, water samples collected after 2, 5, 10, 15 and 20 min. after the start of the backwash and filter media samples collected after the backwash. Facility 3 (Binderup Dambrug) and 4 (Atlantic Sapphire) provided additional samples after a request presented in a newsletter from Danish Aquaculture. The samples from facility 3, an outdoor freshwater trout farm, were collected in June 2020, from two different systems and at different parts of the systems by the manager of the farm. Sample 1 was collected after moving bed biofilter (system 1), whereas the samples 2 and 3 were collected respectively after fixed bed but before moving bed filter and after moving bed filter (system 2). The samples from Facility 4, an indoor salmon farm, were collected at different locations of the system by the manager of the farm.

Measured variables and sample analysis: All samples were analyzed for the large phytoplankton (mainly diatoms), numbers of protozoans, zooplankton and invertebrates, using an inverted microscope for smaller organisms (e.g., protozoans) and a stereo microscope for zooplankton and invertebrates. For facilities 1 and 2, small and abundant organisms such as protozoans and eggs were counted from sub-samples of a few mL, whereas less abundant zooplankton and invertebrates were counted from larger fractions of the sample. In general, ≥ 200 individuals were counted for each sample, although for facilities 3 and 4, the samples contained less individuals, and the minimum of 200 individuals was not always reached. The abundances were calculated based on the sampled volume, volume of the sub-sample and the count, and expressed as cells or individuals 0.1-1 L⁻¹. The differences in total abundances of organisms or abundances of specific groups were tested for differences between the types of the filter media, water and filter samples, systems, sampling times and facilities using a 1-way analysis of variance (ANOVA), or if the assumptions were not met, Kruskal-Wallis ANOVA on ranks.

In facility 1, samples were also analyzed for microbial respiration, algal pigments and chemical oxygen demand (Table 5.1.1). Microbial respiration was measured using an UNISENSE microrespiration system, consisting of a 2-mL airtight chamber that was sub-merged in water bath, an oxygen electrode that was inserted to the chamber through a hole in the lid, a magnetic stirrer, an amplifier and a software that registered the oxygen concentration at 2 s intervals (www.unisense.dk). Each measurement lasted for ≥ 7 min., and the slope of the decrease in oxygen concentration as a function of time was used to estimate the respiration rate.

Algal pigments were measured using AlgaeOnlineAnalyzer fabricate, produced by BBE Moldaenke (<https://www.bbe-moldaenke.de>). This analyzer measures the concentration of green algae, bluegreen algae (cyanobacteria), diatoms, cryptophytes and yellow substance based on their pigments by measuring absorbance at different wavelengths, at intervals of 10 s. An average of the concentrations measured after the initial 2 min. of the measurements were used. COD was measured from the filter samples preserved in sulfuric acid. Samples were placed on a magnet stirrer for 60 minutes at 1800 rpm, in a 1000 ml conic flask, to release biofilm and organisms. After the stirring, the filter media was removed, and filter chips were counted. Each type of filter media had a specific surface area (SSA) per filter chip, given by the manufacturer. The supernatant was measured for total COD using a Hach Lange test-kit (<https://dk.hach.com/>) in duplicates. The COD range of the test kit was 5-60 mg l⁻¹. The details of the sampling and sample analysis for the facility 1 are given in Smith (2019).

TABLE 5.1.1. Description of the facilities for biofilter samples, types and numbers of samples and the measured variables.

Facility	Gamst Aquakultur	Løjstrup Dambrug	Binderup Dambrug	Atlantic Sapphire
Farmed species	Pike perch	Trout	Trout	Salmon
Conditions	20-22 °C; brackish water; indoor	Freshwater; outdoor	Freshwater; outdoor	
Filter type	Fixed and moving bed	Moving bed	Fixed and moving bed	
Sample frequency	April-May 2019; weekly samples in relation to backwash events	Nov 2018; 0-20 min. after backwash	June 2020	June 2020
Sample type	Water, filter media, backwash water (1 system, 3 types of filter media)	Water and filter media (2 systems)	Water (2 systems, different locations)	Water
Measurements	Algal pigments, COD, microbial respiration, zooplankton and macroinvertebrate abundance	Zooplankton and microinvertebrate abundance	Zooplankton and microinvertebrate abundance	Zooplankton and microinvertebrate abundance

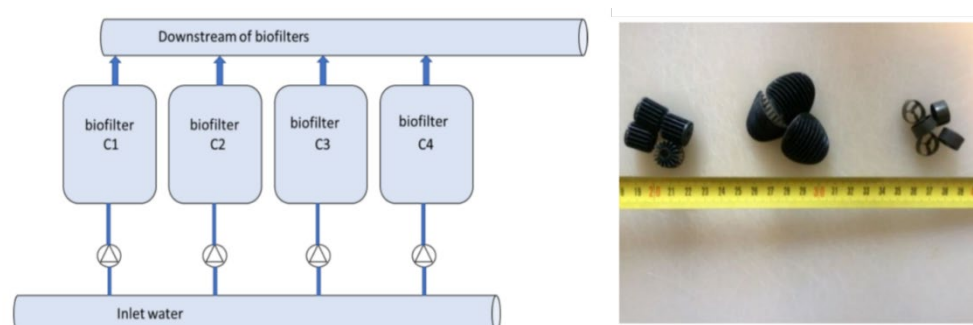


FIGURE 5.1.1. Schematic illustration of the four biofilter systems and the photos of the three types of filter media used in the biofilters 1, 3 and 4 that were sampled here. The specific surface areas of the filter media in C1, C3 and C4 were, respectively 750, 700 and 850 m² m⁻³. The illustrations are from Smith (2019), showing commercial biocarriers from RK Plast, KSK Sadlechip and an unnamed, respectively.

5.2 Biological control

5.2.1 Experiments with cladocerans

Sampling and cultures: Cladocerans were collected in early autumn 2019 from nine lakes situated in the greater Copenhagen area (Table 5.2.1, Fig. 5.2.1). Cladocerans were collected from the shore of the lakes, by filtering >50 L of water onto hand-held nets with 50 µm mesh size, which were subsequently submerged to ca. 5L of lake water and transported to the laboratory within a few hours. In the laboratory, ca. 100 individual cladocerans from lakes where they were abundant were transferred to 2-5 L buckets with 0.2 µm filtered lake water to start a culture that could provide cladocerans for the feeding experiments. This resulted in five cladoceran cultures, whereas four lakes were dominated by copepods and had low cladoceran abundances. The cultures were kept at 18 °C in the 12:12 h cycle of dim light and dark, and fed three times a week with the green alga *Selenastrum capricornutum* in excess (> 400 µg C L⁻¹; assuming a carbon content of 5.6 pg cell⁻¹ for *S. capricornutum*). Cultures of *Daphnia magna* and *Daphnia carinata* were kept in similar way. *D. magna* originated from a pond in south Sweden and was obtained from Dr. S. Hylander in Kalmar University, whereas *D. carinata* was bought from an aquarium web shop in Australia and hatched from dried eggs. *S. capricornutum* culture was obtained from DTU Environment (1st batch) and Copenhagen University (2nd batch), and grown at the temperature of 18 °C and light:dark cycle of 16: 8 h. *Microcystis aeruginosa* (strain CCMP3462) culture was obtained from the NCMA at Bigelow Laboratory culture collection, and grown under similar conditions as *S. capricornutum* culture. Both cultures were diluted frequently to keep the algae in an exponential growth phase.

Sample analysis: In addition to cladoceran sampling, 100 mL of the lake water was preserved in lugol to investigate for the presence of cyanobacteria, 1L was collected for chlorophyll-*a* analysis and ca. 100 mL were frozen at -80 °C for toxin analysis. Replicate 30 mL water samples for chl-*a* were filtered onto GF/F filters and frozen until analysis. Immediately upon the arrival to the laboratory, triplicate samples of 8-35 individual cladocerans (depending on the size of the individuals) were prepared for the analysis of microcystin, one of the main cyanobacterial toxins. The cladocerans were concentrated into Petri-dishes, individuals were picked out with a pipette, flushed two times in clean milli-q water, pipetted into sterile Eppendorf tubes and frozen at -80 °C. The microcystin concentration of *Microcystis aeruginosa* culture was measured after filtering triplicate samples of 40 mL of undiluted culture to GF/F filters that were frozen at -80 °C until analysis. At the same time, the cell concentration was measured under inverted microscope, using Sedgewick rafter counting chambers. Phytoplankton samples were observed for the presence of cyanobacteria colonies and filaments under an inverted microscope. Chl-*a* was measured after 24-h extraction in acetone using a fluorometer, and toxin samples were analysed using ELISA kits (<https://www.epa.gov/ground-water-and-drinking-water/detection-methods-cyanotoxins>). All the sampled lakes had a high chl-*a* concentration and contained visible cyanobacteria filaments or colonies. The microcystin concentrations in the lakes ranged from 0.07 to 0.27 µg L⁻¹ (Table 5.1.2).

TABLE 5.1.2. Name, location and chlorophyll-*a* and microcystin concentrations ($\mu\text{g L}^{-1}$) of the sampled lakes, and body length (μm) and microcystin content ($\mu\text{g ind.}^{-1}$) of the cladocerans used in experiments. Samples for zooplankton toxins were only collected from the lakes where cladocerans dominated. All lakes had a brown-green color typical for cyanobacteria (Fig. 5.1.1) and the presence of cyanobacteria filaments or colonies were later confirmed by microscopic identification. (D) *Daphnia* spp., (B) *Bosmina* spp. (-) No samples, (MD) missing data.

#	Name	Location	Water		Cladocerans	
			Chl- <i>a</i> ($\mu\text{g L}^{-1}$)	Microcystin ($\mu\text{g L}^{-1}$)	Microcystin ($\mu\text{g Ind.}^{-1}$)	Size (μm)
1	Fuglevad st., pond	55°47'01.4"N 12°29'45.7"E		0.16		
2	Mølleå river	55°46'51.8"N 12°26'45.9"E		0.26	0.18 ± 0.06	530 ± 83
3	Lyngby Lake	55°46'30.4"N 12°28'12.7"E		0.24		
4	Vangede Lake	55°44'47.3"N 12°31'11.2"E		0.08	0.07 ± 0.02 (D), 0.06 ± 0.02 (B)	447 ± 288
5	Utterslev mose Lake	55°43'06.8"N 12°30'51.4"E		0.08	0.07 ± 0.02	897 ± 343
6	Damhus Lake	55.677509, 12.484784		0.12		
7	Frederiksberg garden, fountain	55°40'21.9"N 12°31'50.4"E	1.5	0.27	0.08 ± 0.02	774 ± 527
8	Gentofte lake	55.752976, 12.533777	5.6 ± 0.9	0.27	MD	
9	Botanical garden, pond	55.686966, 12.574203		0.07	MD	332 ± 22



FIGURE 5.1.2. Some of the sampled lakes with characteristic coloration of a cyanobacterial bloom.

Feeding experiments: Clearance and ingestion rates of *Daphnia magna*, *Daphnia carinata* and cladocerans collected from five lakes on the toxic cyanobacteria *Microcystis aeruginosa* were estimated in 24-h bottle incubations, with the exception of *D. carinata* where the experiments were continued for 3 days to investigate whether the feeding rates change when the cladoceran acclimatizes to cyanobacteria. Food suspensions containing ca.

400 $\mu\text{g C L}^{-1}$ ($482 \pm 53 \mu\text{g C L}^{-1}$) of the cyanobacteria or ca. 300 $\mu\text{g C L}^{-1}$ ($313 \pm 117 \mu\text{g C L}^{-1}$) of the green alga *Selenastrum capricornutum* (control) were prepared, and their concentrations were measured using a fluorometer (Turner design) and by manual microscope counts using Sedgewick rafter counting chambers. The manual microscope counts were used to estimate the food concentrations at the start of the experiments, assuming a carbon content of 5.6 pg for *S. capricornutum* and 3.2 pg for single *M. aeruginosa* (Yamaguchi et al. 2017).

The food suspensions were divided into four replicate 0.6L bottles for each cladoceran species and four replicate bottles without cladocerans, to estimate the increase in the algae in the absence of grazers. Five to six individual cladocerans were added to the bottles that were then closed air-tight and placed to incubate in a plankton wheel turning ca. one round per minute. After ca. 24-h, the bottles were opened and 20 mL were removed for fluorescence measurements. With the exception of *D. carinata* experiments, rest of the bottle contents were carefully filtered onto 50 μm nets and flushed to Petri-dishes. The condition of the cladocerans (dead / alive and active) were noted, a drop of lugol was added to the Petri-dishes, and the body lengths of cladocerans were measured using a binocular microscope with a precision of 19 μm . *D. carinata* individuals were transferred to new food suspension, prepared in a similar way as the previous day, using a large-mouthed pipette, and only measured after the third day of the experiment.

The clearance and ingestion rates were calculated according to Frost (1972), based on the decrease in raw fluorescence in the bottles without cladocerans compared to bottles containing cladocerans, using a fluorometer. To get the ingestion rate in carbon, the raw fluorescence was transferred to numbers of cells using the average cell count to fluorescence ratio from all samples where both the cell counts and fluorescence were measured (raw fluorescence: cell concentration (cells mL^{-1}) 0.0011 for *S. capricornutum* and 0.0041 for *M. aeruginosa*). For the weight-specific ingestion, the body lengths of cladocerans were converted to carbon using the length to carbon conversions from Vasama & Kankaala (1983); the ratio for *Daphnia* spp. was used for the large cladocerans whereas the ratio of *Bosmina* spp. was used for small cladocerans.

5.2.2 Experiments with biofilter organisms

Cultures: Unidentified ostracods and harpacticoid copepods for experiments were collected from the biofilter in facility 1, and kept on 5-L containers with < 0.2 μm filtered lake water and a mixture of the three types of filter media (Fig. 5.1.1). Cultures were fed with *Selenastrum capricornutum* in excess concentration, and kept under similar conditions as the cladoceran cultures.

Feeding and reproduction experiments: To estimate the potential feeding and reproduction rates of ostracods and harpacticoids from the biofilter community, 3-day laboratory experiments were conducted with these species. In day 1, 5-6 ostracods and 8-10 harpacticoid copepods of approximately similar size were placed into five replicate 250-mL bottles containing *Selenastrum capricornutum* with an average concentration of 340 $\mu\text{g C L}^{-1}$ and five replicate control bottles without zooplankton were set up to estimate the algal growth in the absence of grazers. The experiments were carried out similar to the 3-day experiment with *D. carinata* (see above), with an exception of daily egg counts that were conducted using a binocular microscope, after filtering the suspension onto 20 μm nets and flushing it to Petri-dishes. The carbon content of ostracods was estimated from the length based on the dry weight to length regression of Anderson et al. 1998; the carbon content of harpacticoids was estimated from the length based on Longsdale & Levinton 1985.

Effect of nutrients, propagule size and ostracod grazing on algal growth: To estimate the interaction between nutrient concentration, propagule size and ostracod grazing on the growth rate of *Selenastrum capricornutum*, we designed an experiment with three different ammonium levels (10, 70 and 700 $\mu\text{g NH}_4 \text{L}^{-1}$), three different start concentrations of *S. capricornutum* (5000, 25 000 and 50 000 cells mL^{-1}) and three different concentrations of ostracods (0, 1 or 5 individuals per 250 mL incubation bottle). Experiments were conducted in <0.2 μm filtered lake water that had start nutrient concentrations of 4.6 $\mu\text{M PO}_4$, 30.4 $\mu\text{M NO}_3$ and 1.6 $\mu\text{M NO}_2$. Each treatment was run in triplicates and had a duration of seven days, with measurements of raw fluorescence conducted in the intervals of 1-2 days.

5.3 Physical control: Experiments with protein skimmers, ozone and hydrogen peroxide

Two experiments with foam fractionators (protein skimmers) and using water from recirculating aquaculture systems were conducted: Experiment 1 investigated the effects of foam fractionation (FF) with and without addition of hydrogen peroxide and salt using 36 individual 6-h batch trials, whereas experiment 2 investigated effects of foam fractionation (FF) and ozone using 12 individual 800 L pilot scale RAS units that were run for 8 weeks. Turbidity, particle concentrations and microbial activity were used as indicators of the water quality.

Experiment 1 - Foam fractionation combined with H₂O₂ and salinity: The experimental design included 3 factors: Presence or absence of FF, addition of H₂O₂ (0 or 10 mg L⁻¹ H₂O₂) and addition of NaCl (salinity of 0, 3 or 10 ppt; Fig. 5.3.1). Salinity was adjusted by adding sea salt in the RAS water before it was transferred into the 30 L experimental tanks. Each tank was equipped with a pump for homogenous mixing of the water, a foam fractionator, and an online turbidity meter. The FF used was 28 cm high, and the air was supplied from the bottom using a wooden air stone. The foam produced by the FF was collected by overflow into plastic bottles.

Each experimental trial lasted for 6 hours; samples were collected prior to treatment and at the end of the trial. The measurements included turbidity, particle numbers for the size range of 5.6-160 µm in diameter, total suspended solids (TSS) and total chemical oxygen demand (COD_{Total}) for the organic matter that was removed from the water by FF as foamate. After measuring TSS, and COD_{Total} in the foamate and initial water, the percent removal efficiency of FF for these two-variables was calculated according to:

$$RE (\%) = 100 \times \frac{C_f \times V_f}{C_0 \times V_W} \quad (1)$$

where *RE* is the removal efficiency (%), *C_f* is the concentration (TSS or COD_{Total}) in the foamate, *V_f* is the total foamate volume (L), *C₀* is the initial concentration (TSS or COD_{Total}) in the water, and *V_W* is the initial water volume (L). Microbial activity was quantified using the hydrogen peroxide (H₂O₂) decomposition assay as an expression of microbial activity (Pedersen et al., 2019). This assay relies on the quantification of the enzymatic degradation of H₂O₂, which is calculated as a degradation rate constant *k* based on:

$$C_t = C_0 \times e^{-kt} \quad (2)$$

where *C_t* is the H₂O₂ concentration at time *t* and *C₀* is the nominal H₂O₂ concentration at time 0.

Particle concentration, turbidity and bacterial activity were normalized as percent of measurements at time zero to facilitate a comparison between treatments at the end of the experiment and to correct for differences in the starting conditions of replicated experiments. A three-way ANOVA was conducted to compare the main effects of foam fractionation, hydrogen peroxide and salinity, as well as interaction effects. Differences in treatment means were tested by Tukey's post-hoc test with a pre-defined significance level of *p* < 0.05. Statistical analyses were processed using SPSS version 25 and Microsoft Excel. Further details are described in Jafari, 2020.

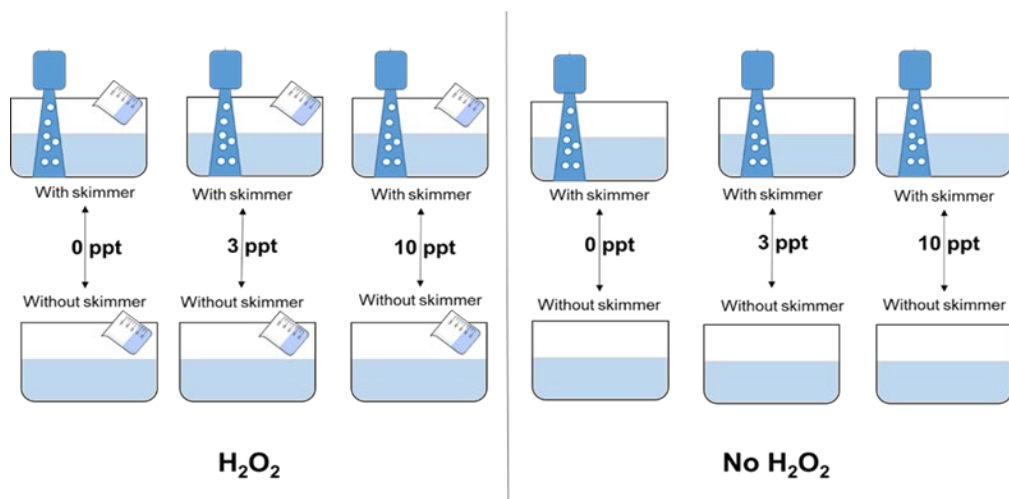


FIGURE 5.3.1. 3-factorial design of Exp. 1. Each treatment ($n=12$) was conducted in triplicates in 30-L containers with 20 L RAS freshwater ($N=36$).

Experiment 2 - Foam fractionation combined with Ozone: A two-by-two factorial experiment with foam fractionation and ozonisation as main factors was performed in 12 replicated, 0.8 m³ pilot scale freshwater RAS. Four treatment combinations were applied: Three control RAS without FF or O₃, three RAS with FF (FF), three RAS with O₃ dosing (O₃), and three RAS with FF + O₃ dosing combined (FF+O₃). Each RAS was composed of a 100 L cylindroconical biofilter filled with 40 L RK BioElements (RK BioElements, Denmark) with a specific surface area of 750 m² m⁻³ and operated as a moving bed biofilter with an air flow of 4 L min⁻¹, a 200 L pump sump and a 500 L cylindroconical rearing tank with a metal grid preventing fish from assessing the bottom cone, which contained a 0.8 L waste collector/settling column (Fig. 2.3.2). Two DC Runner 5.2 pumps pumped approximately 1500 L h⁻¹ to the biofilter and 2000 L h⁻¹ to the rearing tank, corresponding to a retention time in the rearing tank of approximately 15 min.

In order to test the effects of FF and O₃, six systems were fitted with foam fractionators, three systems were fitted with 1.8 m high bubble columns (same height as the FF) where O₃ was injected and the remaining three systems were kept standard as control systems. Three of the systems fitted with FF were supplied with O₃ (injected in the skimmer), while the remaining 3 systems were feed only air, to test the effects of FF alone. Three ozone generators were used to supply O₃. In order to mitigate small changes in O₃ production, each ozoniser supplied a system fitted with a bubble column and a system fitted with a FF. Foam fractionators were operated with a water flow rate of 1500 L h⁻¹ and an air flow rate of either 1320 L h⁻¹ (air alone) or 1200 L h⁻¹ (air) plus 120 L h⁻¹ ozonized air. Bubble columns were supplied with 120 L h⁻¹ ozonized air. Hydraulic retention time within FF and bubble columns was kept equal to ensure equal contact time in both systems. All gas intakes were controlled by flow meters. Ozone was injected at a dosage of 20 g O₃ kg⁻¹ feed (83 mg O₃ h⁻¹). Incoming O₃ gas concentrations were measured using a UV spectrophotometer (at 254 nm) and flow through cell as described in Hansen et al. (2010). Furthermore, to estimate the amount of O₃ that reacted in the water, O₃ gas concentrations leaving the foam fractionators and bubble columns outflow air were measured at regular intervals.

Each system was stocked with a total biomass of 8.05 ± 0.03 kg juvenile rainbow trout (*Oncorhynchus mykiss*). The fish were fed a fixed amount of 100 g d⁻¹ (Efico E 920, Biomar, Denmark), and 60 L of water was replaced each day, resulting in a feed loading of 1.66 kg feed m⁻³. Oxygen levels ranged between 85 and 90% saturation throughout the trial. Sodium bicarbonate was added when needed to keep pH between 7.0 and 7.3. Primary solids were collected in settling columns at the bottom of the tanks. Each day, the conical part of the tanks was cleaned using magnetic cleaners and the settling columns were emptied. The trial lasted eight weeks and samples were obtained once a week. All 12 RAS had been operated under similar conditions without foam fractionators or ozone for 13 weeks prior to the trial, fed 60 g daily, and all biofilters were fully operational. Feeding was increased from 60 to 100 g 3 days prior to the start of the trial. Fish biomasses were weighed at the start and by the end of the trial.

Water samples were collected on day 0 prior to starting the foam fractionators and ozonisers, and every day before the daily routines. A 5 L water sample was collected from the sump of each RAS and split into homo-

geneous subsamples for individual analysis. pH was measured daily in the sump before daily routines, and temperature was logged automatically. Particles of 1-200 μm were measured using a Coulter Counter with both 50 μm and 280 μm apertures. Total particle number (PN), total particle volume (PV), and total particle surface area (PSA) for the full range measured (1-168 μm) were calculated by summing the contribution from the different size classes. To compare systems, particle size distributions were summarized by the β value as described by Patterson et al. (1999). In short, β value is the slope of the log-log transformed relationship between number of particles within size classes and the corresponding size class median diameter. A low β value indicates a system dominated by larger particles whereas a high β value indicates a system dominated by smaller particles.

Turbidity was measured using a hand-held turbidometer, while the total UV radiation was measured using a UV spectrophotometer and measuring percentage transmission in quartz cuvettes at 254 nm. Microbial activity was quantified using the hydrogen peroxide degradation assay as described before, and the BactiQuant assay, which expresses the microbial activity as relative BQ values. Organic matter concentration was estimated as 5-days biological oxygen demand (BOD_5) and chemical oxygen demand (COD). Both metrics were measured in non-filtered ($\text{BOD}_{5\text{-Tot}}$ and $\text{COD}_{\text{-Tot}}$) and 0.45 μm filtered ($\text{BOD}_{5\text{-Diss}}$ and $\text{COD}_{\text{-Diss}}$) water samples. Corresponding particulate fractions ($\text{BOD}_{5\text{-Part}}$ and $\text{COD}_{\text{-Part}}$) were calculated as the difference between the non-filtered and the filtered samples. Nitrate-N, nitrite-N and ammonium-N were measured by spectrophotometry. Eight bio-elements from each biofilter were collected weekly and placed dry in 50 mL test tubes that were stored at -20°C prior to COD analysis. To detach the organic matter, 20 mL Milli-Q water was added to each test tube and the tubes were sonicated for 10 min using an ultrasonic cleaner. The resulting water was transferred to a beaker and analyzed for $\text{COD}_{\text{-Tot}}$ as described above. Ozone concentrations in the water were measured using both the colorimetric method (Buchan et al. 2005; Schroeder et al. 2015) and the indigo method.

Results of the two main factors (i.e., foam fractionation and ozonation) were compared using data from the last three trial weeks ($n = 9$), to account for the weekly variability in the system. Data were tested for normality (Shapiro-Wilk test) and equal variance (Brown-Forsythe). Data that did not meet these requirements were log transformed. A two-way ANOVA analysis followed by a Holm-Sidak analysis was conducted in case of significant ($p < 0.05$) main effects. As BactiQuant and $\text{BOD}_{5\text{-Diss}}$ results did not meet the equal variance assumption either before or after conversion they were not subjected to two-way ANOVA analysis. Removal percentages were calculated relative to the control treatment based on averages of the last three trial weeks. Statistical analyses were performed in SigmaPlot 13.0 (Systat software Inc., USA).

TABLE 5.3.1. A) Equipment used in the set up of the RAS, and B) methods and equipment used to analyze the water quality.

Variable	Method and Producer
A. RAS set up	
Pump	Tunze Silence 1073.008, Tunze Aquarientechnik GMBH, Germany
Foam fractionator	Delaman® Protein Skimmer para Acuario Marino, size No1, MN-27220-SE1, Amazon
Air stone	Sander No. 2, Erwin Sander Elektroapparatebau GmbH, Germany
Pump	DC Runner 5.2 pumps; Aqua Medic GmbH, Bissendorf, Germany
Foam fractionator	Sander Fresh Skim 200, Erwin Sander Elektroapparatebau GmbH, Germany
Ozone generator	Ozonizer S 500, Erwin Sander Elektroapparatebau GmbH, Germany
Flow meter	Key Instruments; Variable area flow meter, Key Instruments, USA
Magnetic cleaners	Tunze care magnet, TUNZE® Aquarientechnik GmbH, Germany
B. Water quality	
pH	Hach HQ40d Portable Multi Meter, Hach Lange, USA
T	OxyGuard Pacific system; OxyGuard International A / S, Denmark
Oxygen	OxyGuard Pacific system; OxyGuard International A/S, Denmark
Turbidity	Online; Solitax LXV423.99.10000, Hach, United States
Turbidity	Hand-held; Hach 2100Q, Hach, United States
Particle counts	Coulter counter; Multisizer 4e Coulter Counter, Beckman Coulter Life Science, US
Chemical oxygen demand (COD)	ISO 6060 (1989)

Suspended solids	APHA standard method (2005) ¹
Microbial activity	Enzymatic degradation of H ₂ O ₂
Microbial activity	BactiQuant; Mycometer A/S, Denmark
UV light	UV spectrophotometer ² ; Beckman DU® 530 Life Science UV/Vis Spectrophotometer, Beckman Coulter, Inc, Indianapolis, USA
Biological oxygen demand (BOD)	ISO 5815:1989 modified by adding allylthiourea; Fluka Chemika
Nitrate-N, nitrite-N and ammonium-N	ISO 7890-1 (1986), DS 223 DS and DS 224
Ultrasonic cleaner	Branson Ultrasonics Corp, USA
Ozone	N,N-diethyl-p-phenylenediamine ³
Ozone	Ozone AccuVac® Ampules, Hach Lange, USA

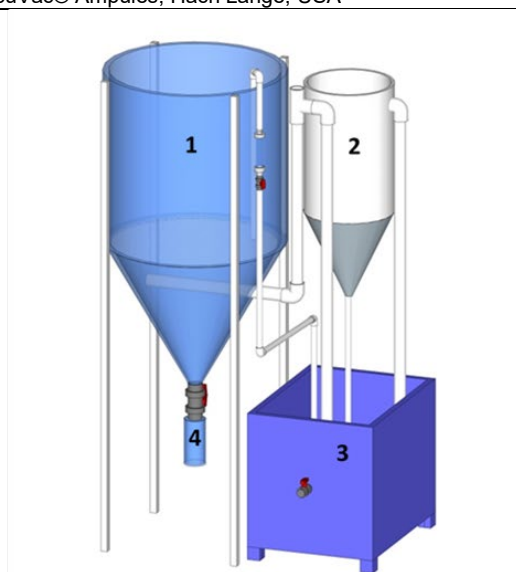


Fig. 2.3.2. Pilot scale RAS including a: 1) Rearing tank, 2) moving bed biofilter, 3) pump sump and 4) sludge collector

5.4 Survey

To understand the aquaculture farmer's perception of barriers related to uptake of new innovations or technology a survey was developed, structured around the key aspects presented by Kumar et al. (2018). The method followed a two-step approach containing firstly an online survey targeting all European aquaculture farmers (App. 2), and a semi-structured interview of Danish aquaculture farmers, similarly structured around keywords and challenges suggested by Kumar et al. (2018) (App. 3). The online survey was included in light of the international nature of aquaculture production, EUs inner market, and the presence of European aquaculture R&I research programs.

European scale: The online survey was performed using the free software Google Forms, and distributed through established national and European aquaculture industry networks such as Federation for European Aquaculture Producers (FEAP), Dansk Akvakultur and other contact points known by the research groups, to provide as large a number of respondents as possible. Open ended questions for each of Kumar's categories were formulated, enabling the further analyses to categorize answers down to "sub-category" using the presence of keywords related to the specific subcategories (i.e. mentioning of ownership, relevance of location, availability of funding etc.). The survey was launched 5.6.2020 and closed 1.9.2020.

¹ Pedersen et al. 2019

² Hansen et al. 2010

³ Buchan et al. 2005, Schroered et al. 2015

Danish scale: The semi-structured interviews with seven Danish fish farmers took place throughout September 2020. Fish farmers working with freshwater pond systems were particularly targeted as this is the most common production system in Denmark (<https://www.statistikbanken.dk/10207>). Based on the total number of freshwater pond-based systems (160, in 2017), the number of respondents translated to 4.3% of the producers, though this number is a low estimate as some of the respondents were in charge of running several individual farms. In this respect, and taking into account that none of the respondents declined to answer the questions, we consider the responses representative for the sector.

Due to the COVID-19 situation all interviews were performed in Danish over the telephone and the number of questions were adjusted, so that the length of the interviews was approximately 15-20 minutes. To ensure that the farmers were able to provide meaningful answers to generic questions, it was decided to formulate questions addressing their actual experiences, rather than using hypothetical examples. The questions can be seen in appendix 3. Similar to the online survey, the approach was designed to cover the categories highlighted by Kumar et al. (2018).

6. RESULTS AND DISCUSSION

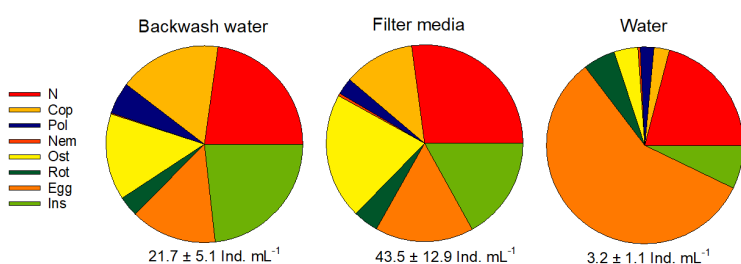
6.1 Biofilter communities and metabolic activity

Main results:

- Biofilters harbor a rich community of protozoans and invertebrates such as copepods, ostracods, nematodes, polychaetes, rotifers and diverse eggs with abundances $> 20\,000$ individuals L^{-1}
- Most of these organisms are associated with the biofilter rather than free-floating in the water
- The proportional importance and abundances of different organisms varies between the facilities, likely due to the differences in the salinity and light conditions, but are similar between different systems in the same facility and not influenced by the type of the filter chips
- Abundances of most organisms do not seem to change due to maintenance cycle, and they are likely to remain in the system irrespective of the backwashing
- Biofilter communities include organisms from different trophic levels and development stages, as well as organisms with high feeding rates. Therefore, biofilters might function as small ecosystems with active reproduction and predator-prey interactions and high turnover times.

Biofilter communities: Biofilters in all facilities harbored an abundant community of protozoans, zooplankton and invertebrates, although the total concentrations and dominating groups differed between the facilities (Fig. 3.1.1). The organisms that were abundant in most samples included different life-stages of copepods, polychaetes and rotifers, whereas ostracods were mainly abundant in the facility 1, and nematodes in the facility 2. Also, facility 1 had high concentrations of insects and mites, whereas facility 2 had more single-celled organisms, most likely diverse protozoans. Facilities 3 and 4 mainly had nematodes, protozoans and early life stages (eggs) of zooplankton. However, the sampled volumes in these facilities were smaller, which could have resulted in underestimation of invertebrates in the samples. The average abundances of all organisms were extremely high, ranging from 3 to 44 ind. mL^{-1} in the facilities 1, 3 and 4, and from 260 to 370 ind. mL^{-1} in facility 2. The high concentration in facility 2 was not only due to the high concentrations of protozoans, but also the abundance of easily identified zooplankton was high at 93 ± 16 ind. mL^{-1} . For comparison, maximum concentrations of zooplankton in shallow productive lakes are around 10-20 ind. mL^{-1} . Most biofilter samples exceeded these concentrations.

A. Facility 1



B. Facility 2

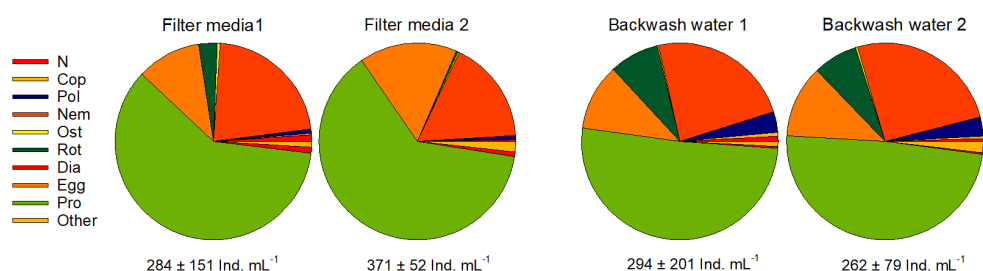


Fig. 6.1.1. Community composition (% of the total abundance) of protozoans, zooplankton and invertebrates in the backwash water, filter media and water before the backwash in facility 1 (upper panel), and in filter media and backwash water in the two systems of the facility 2 (lower panel). (N) Copepod nauplii, (Cop) copepods, (Pol) polychaetes, (Nem) nematodes, (Ost) ostracods, (Rot) rotifers, (Egg) unidentified eggs, (Ins) insect larvae, (Dia) diatoms, (Pro) Protozoans, (Other) other organisms.

The abundance of different groups were different between the facilities and between water, biofilter and backwash water samples (Fig. 6.1.1), but not between the different systems in the same facility or between different types of filter chips (1-way ANOVA; $p > 0.05$; App. 4). Typically, most organisms were associated with the filter media, and got suspended during the backwash. The concentrations in water before the backwash were lower, and the species composition different from the filter media and backwash samples (Fig. 6.1.1). In the weekly samples, the abundances of some organisms as well as the total abundances on the filter media samples dropped slightly after the backwash, but returned to the original levels soon after (Fig. 6.1.2a, App. 5 and 6). In the short-term samples, the organisms were flushed from the filter to (backwash) water, but remained in the system (Fig. 6.1.2b, App. 6). There was thus no indication that the backwash would have removed zooplankton or invertebrates from the system.

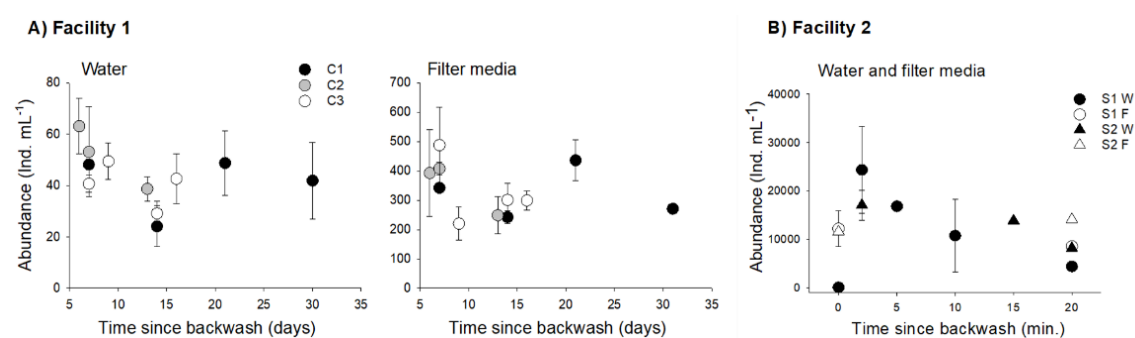


FIGURE 6.1.2. Total abundance of protozoans, zooplankton and invertebrates in the water and in filter media in facility 1, and in the two systems of facility 2, as a function of the time since backwash (Ind. mL⁻¹; mean \pm SD). (C1), (C2) and (C3) refer to the three types of filter media (Fig. 2.1.1.), (S1) and (S2) to the two systems in facility 2, and (W) and (F) to the backwash water and filter media, respectively. Note different scales of the x-axis.

Metabolic activity in the filter: High abundance of copepod nauplii and unidentified eggs in the biofilters indicated an active reproduction of both copepods and other organisms. In facility 2, the nauplii abundance was on average 4.5 (\pm 0.4) times higher than copepodite abundance in the filter media, and 1.2 (\pm 0.2) times higher than copepodite abundance in the backwash water. Egg abundance accounted for 14 \pm 3 and 11 \pm 1 % of the total abundance in filter media and backwash water, respectively (App. 6). In facility 1, nauplii abundance was on average two times higher than copepodite abundance in the filter media and 1.4 times higher than copepodite abundance in the backwash water, and eggs accounted for 14-16 % of the total abundance of organisms in the filter media and backwash water (App. 5). The size range of the organisms ranged from $< 50 \mu\text{m}$ (some of the protozoans, diatoms, eggs) to $> 1 \text{ mm}$ (insect larvae), and included both species that may primarily feed on bacteria and other micro-organisms attached on the biofilter (harpacticoid copepods) and predatory organisms that might feed on the smaller zooplankton (nematods, polychaetes). Biofilter is likely to provide abundant food sources for these different trophic levels. High metabolic activity on the biofilter was also suggested by the respiration measurements, which indicated an average microbial respiration rate of $1286 \pm 99 \mu\text{mol O}_2 \text{ L}^{-1} \text{ h}^{-1}$ in facility 1 (Smith 2019). Also, despite the generally dim light conditions, different algal pigments were abundant in facility 1 (Smith 2019), as were diatoms in facilities 2, 3 and 4.

The feeding experiments demonstrated that particularly biofilter ostracods can obtain high daily feeding and reproduction rates ranging from 3.2 to 10.5 $\mu\text{g C ind.}^{-1} \text{ d}^{-1}$ and from 3.6 to 4.9 eggs $\text{f}^{-1} \text{ d}^{-1}$, respectively (Fig. 6.1.3). These rates correspond to carbon specific rates of 2.5 and 0.8 $\mu\text{g C} (\mu\text{g C})^{-1} \text{ d}^{-1}$, which indicates a high gross growth efficiency (GGE) of 0.7 (Table 6.1.2). Although the exact dates are subject to errors in the length to weight conversions (see methods), they indicate a high production to biomass ratio in these organisms, and therefore potentially fast carbon turnover rate of the biofilter community. Also the harpacticoid copepods had high

weight-specific ingestion rates of $0.42 \mu\text{g C } (\mu\text{g C})^{-1} \text{ d}^{-1}$ (Table 6.1.2), although due to their small size their individual carbon ingestion rates were much lower than the carbon ingestion of ostracods (Fig. 6.1.3). Also, the feeding and reproduction rates of harpacticoids were variable, and particularly the egg production was low (Table 6.1.2). Since the amount of nauplii in biofilters was high, the low and variable reproduction in the experiments could have been a result of experimental conditions, for instance the food species which might not have been ideal for harpacticoids or sensitivity to handling.

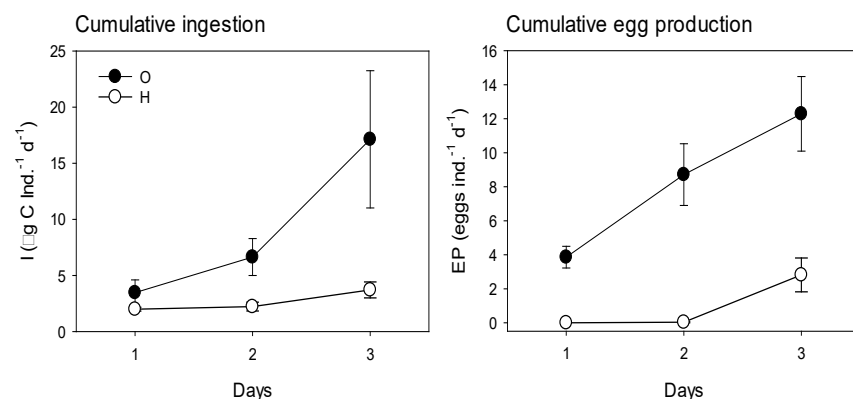


FIGURE 6.1.3. Cumulative ingestion ($\mu\text{g C ind.}^{-1}$) and cumulative egg production ($\text{eggs ind.}^{-1} \text{ d}^{-1}$) of ostracods and harpacticoid copepods collected from the biofilters and fed green alga *Selenastrum capricornutum* (mean \pm SD). (O; filled circles) Ostracods, (H; open circles) harpacticoids.

TABLE 6.1.2. Average size (μm), clearance rate ($\text{mL ind.}^{-1} \text{ h}^{-1}$), weight-specific ingestion and egg production rates ($\mu\text{g C } (\mu\text{g C})^{-1} \text{ d}^{-1}$) and gross growth efficiency of ostracods and harpacticoids in the experiments (mean \pm SD of the three experimental days). (MD) Missing data.

	Ostracods	Harpacticoids
Adult size	481 ± 62	453 ± 30
Egg size	92 ± 13	MD
Clearance	0.56 ± 0.63	0.13 ± 0.14
Weight-specific ingestion	2.5 ± 2.9	4.2 ± 5.0
Weight-specific egg production	0.77 ± 0.41	0.05 ± 0.01
GGE	0.70 ± 0.75	< 0.04

6.2 Interaction of nutrient concentration, propagule size and ostracod grazing on the formation of algal biomass

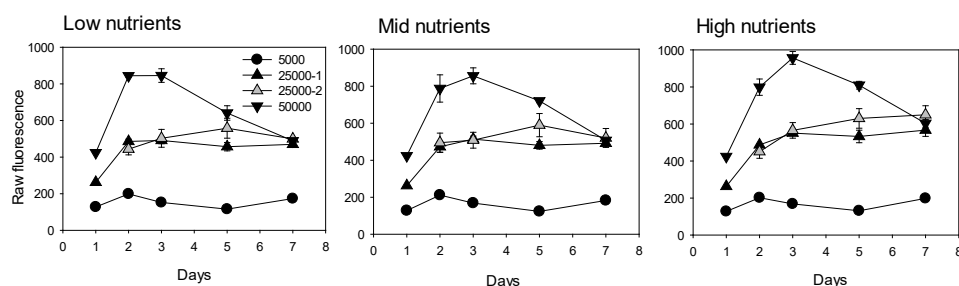
Main results:

- A small propagule size ($\leq 5000 \text{ cells mL}^{-1}$) did not allow for a high population growth, irrespective of the nutrient concentrations
- The effect of nutrients on algal growth depended on the cell density: Population that started with $25\,000 \text{ cells mL}^{-1}$ profited most from increased nutrients, whereas the highest population density of $50\,000 \text{ cells mL}^{-1}$ became limited by other factors, e.g., light (due to shelf-shading)
- Even a low concentration of ostracods was able to effectively reduce algal abundance, irrespective of the nutrient concentration or propagule size

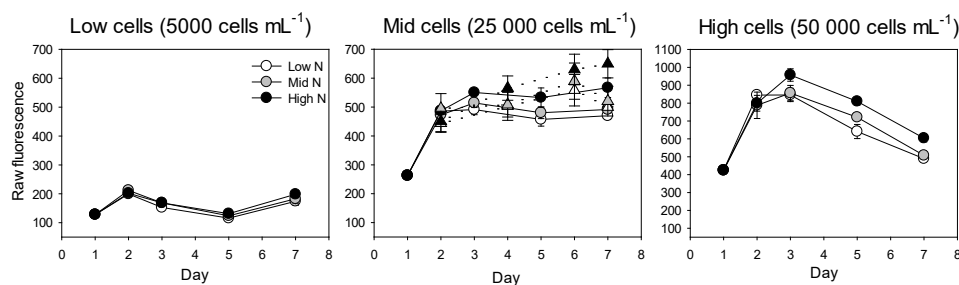
A population with a relatively low propagule size of ≤ 5000 *Selenastrum capricornutum* cells mL^{-1} was not able to increase in abundance, but maintained similar population size irrespective of the addition of nutrients (Fig. 6.2.1a). In contrast, the populations that started with $25\,000$ and $50\,000$ cells mL^{-1} doubled within the first 1-2

days of the experiment in all nutrient treatments. Nutrients influenced the maximum cell concentration, which was highest in the highest nutrient concentration, in day 3 for the population that started with 50 000 cells mL⁻¹ and in day 7 for the population that started with 25 000 cells mL⁻¹, and generally reflected the nutrient concentrations (Fig. 6.2.1b). The effect of ostracod grazing was visible already during the first three days of the experiment, with the largest effect on the lowest nutrient concentrations, for the population with the smallest propagule size and in the experiments with highest ostracod concentration. However, after four days of the experiment the algae concentrations dramatically decreased in all ostracod treatments, resulting in $\geq 80\%$ reduction of the algae concentrations, irrespective of the propagule size, nutrient treatment or ostracod abundance (Fig. 6.2.1c). This demonstrated the ability of ostracods to control the algae abundances, and emphasizes the importance of balanced predator-prey interactions in the biofilter.

A) Effect of propagule size



B) Effect of nutrients



C) Effect of ostracods

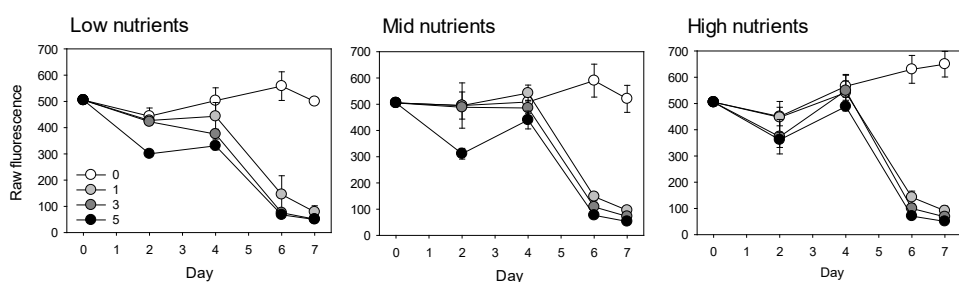


FIGURE 6.2.1. Concentration of *Skelenastrum* sp. as a function of A) propagule size (cells mL⁻¹), B) nutrient addition ($\mu\text{g NH}_4$), and C) amount of ostracods (ind. bottle⁻¹) over seven days of experiments (raw fluorescence; mean \pm SD).

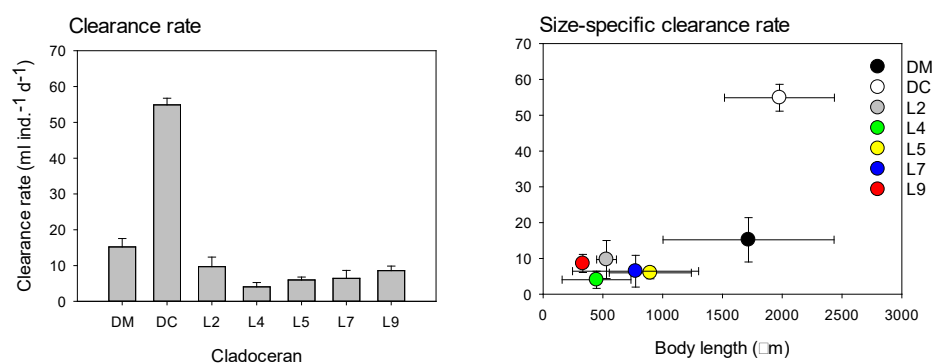
6.3 Biological control of toxic algae

Main results:

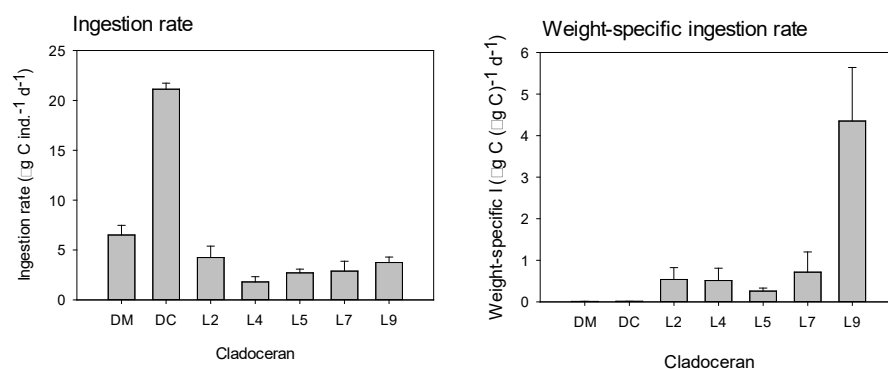
- All tested cladocerans were able to feed on toxic cyanobacteria *Microcystis aeruginosa*
- Organisms that were collected from lakes with abundant cyanobacteria had higher weight-specific ingestion rates than cultured cladocerans that had not previously encountered cyanobacteria
- Cladocerans collected from lakes with cyanobacteria contained cyanobacteria toxins, suggesting that the toxins accumulate in their bodies

Feeding rates on cyanobacteria: All tested cladoceran species were feeding on the cyanobacteria *Microcystis aeruginosa*, with clearance rates ranging from 4 to 55 ml ind.⁻¹ d⁻¹, depending on the size of the cladoceran (Fig. 6.3.1a). The highest clearance and ingestion rates (55 ± 3.7 ml ind.⁻¹ d⁻¹ and 21 ± 1.2 µg C ind.⁻¹ d⁻¹, respectively) were obtained with *Daphnia carinata* that had an average body size of 2.0 ± 0.5 mm, whereas the cladocerans that were collected from Copenhagen lakes had an average body size between 330 and 900 µm, and clearance and ingestion rates that ranged from 4.0-9.7 ml ind.⁻¹ d⁻¹ and 1.8 and 4.2 µg C ind.⁻¹ d⁻¹, respectively (Fig. 6.3.1). When the clearance and ingestion rates were related to body size, the cladocerans collected from the Copenhagen lakes were clearly more effective in feeding on the cyanobacteria, obtaining weight-specific ingestion rates that were up to 4.4 times of their body weight d⁻¹, while the weight-specific ingestion rates of the large cultured *Daphnia* species were around 1% of their body weight d⁻¹ (Fig. 6.3.1b). The weight-specific ingestion rates seemed to be related to the toxin load of the cladocerans, so that the cladocerans that had a high body content of cyanobacteria toxins at the time of sampling, also had high weight-specific ingestion rates (Fig. 6.3.1c). Unfortunately, no microcystin samples were collected from the small cladocerans collected from lake 9 (Botanical garden in Copenhagen) that had the highest weight-specific ingestion rate of *Microcystis*. However, a preliminary experiment showed that it could obtain a body load of microcystin up to 0.79 µg L⁻¹ (Tardum Franzén Storm 2019), suggesting a high tolerance of cyanobacteria toxins in this species. Our results thus suggested that cladocerans that were collected from lakes with abundant cyanobacteria could feed efficiently on these species, and seemed to be resistant to cyanobacteria toxins. It also appeared that all cladocerans from these lakes had microcystin in their body (Table 6.1.2), suggesting that the toxins would not be degraded, and could thus bioaccumulate in the food web.

A) Clearance rate



B) Ingestion rate



C) Ingestion in relation to body toxin content

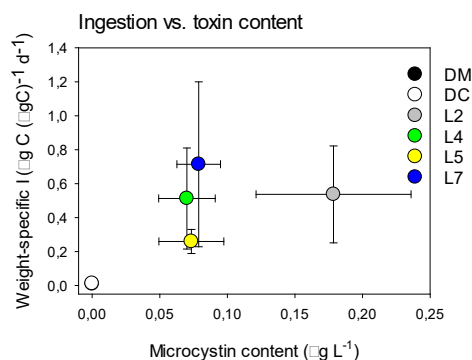


FIGURE 6.3.1. A) Clearance rate ($\text{ml ind.}^{-1} \text{h}^{-1}$), B) Ingestion ($\mu\text{g C Ind.}^{-1} \text{d}^{-1}$) and weight-specific ingestion ($\mu\text{g C } (\mu\text{g C})^{-1} \text{d}^{-1}$) rates and C) weight-specific ingestion rate as a function of the body microcystin content ($\mu\text{g L}^{-1}$) of the cladocerans collected from different lakes; mean \pm SD). (DM) *Daphnia magna*, (DC) *Daphnia carinata*. L2-7 refer to the different lakes as presented in Table 2.1.2.

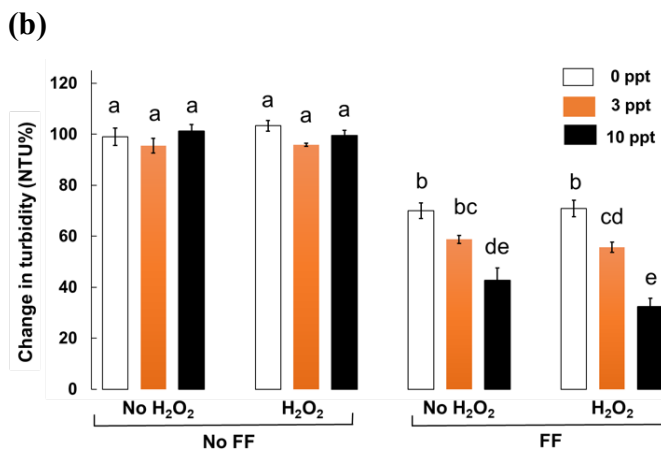
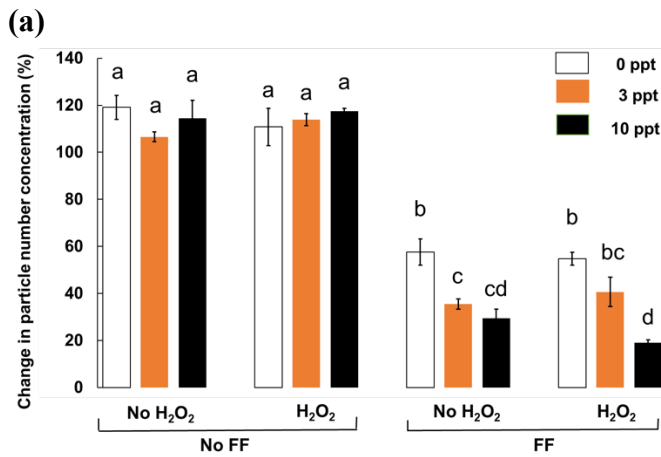
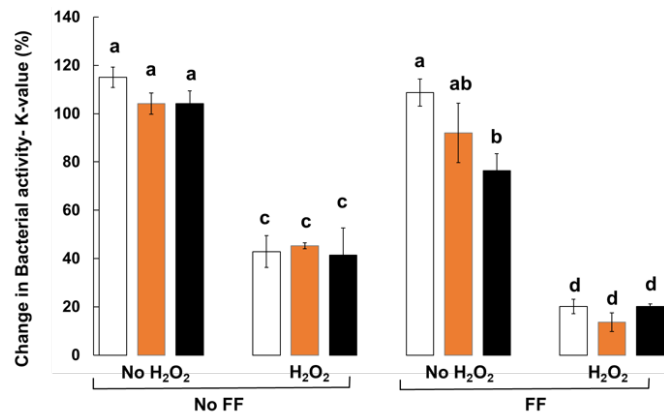
6.4 Physical control

Main results:

- Foam fractionation (FF) was demonstrated to be a simple and effective water treatment technique to remove microparticles from freshwater RAS
- FF in combination with hydrogen peroxide and addition of salt led to significant reduction of bacteria and turbidity in RAS
- Pilot scale RAS trials documented beneficial properties of FF in terms of removal of microparticles, reduction of bacterial load, reduction of biodegradable organic matter (BOD_5) and improvement of water clarity.
- FF combined with ozone led to an immediate and persistent improvement of water quality measured as bacterial load and microparticle concentrations.

6.4.1. Experiment 1 - Foam fractionation combined with H_2O_2 and salinity

The experimental trials with foam fractionation (FF) demonstrated a substantial removal of microparticles and organic matter and a reduction of bacterial activity that resulted in significant improvements in physical and chemical water parameters. FF and addition of H_2O_2 reduced *bacterial activity* both alone and in combination, with additive effects of salinity occurring at 10 ppt. On average, 58% lower bacterial activity was observed in treatments with FF + H_2O_2 compared to the treatments without FF. Similarly, FF reduced *microparticle numbers and turbidity* to levels that were approximately 74 and 45% lower in the FF treatments compared to control treatment without FF, respectively, whereas H_2O_2 did not affect particle numbers or turbidity. Salinity reduced both particle numbers and turbidity with a significant interaction between FF and salinity (Fig. 6.4.1). The organic matter removed from the water and collected as foamate was measured as removal percentage of total suspended solids (TSS) and total chemical oxygen demand (TCOD). Both H_2O_2 and salinity increased the removal of TCOD and TSS. However, in the H_2O_2 treatment with 10 ppt, respectively 15 and 20% more TSS and TCOD were removed compared to the treatment without H_2O_2 (Fig. 6.4.2).



(c)

FIGURE 6.4.1. Relative changes after 6 hours of treatment (% from the start values), in a) bacterial activity, b) particle concentration, and c) turbidity. Treatment include foam fractionation (FF) vs. control (no FF); hydrogen peroxide (10 mg l⁻¹ H₂O₂) vs. control (no H₂O₂) and salinity (0, 3 or 10 ppt). The data presented (mean ± SE; n=3) reflects normalized value calculated according to each group's initial values before treatment (t=0). The different low cap letters denotes significant differences between groups.

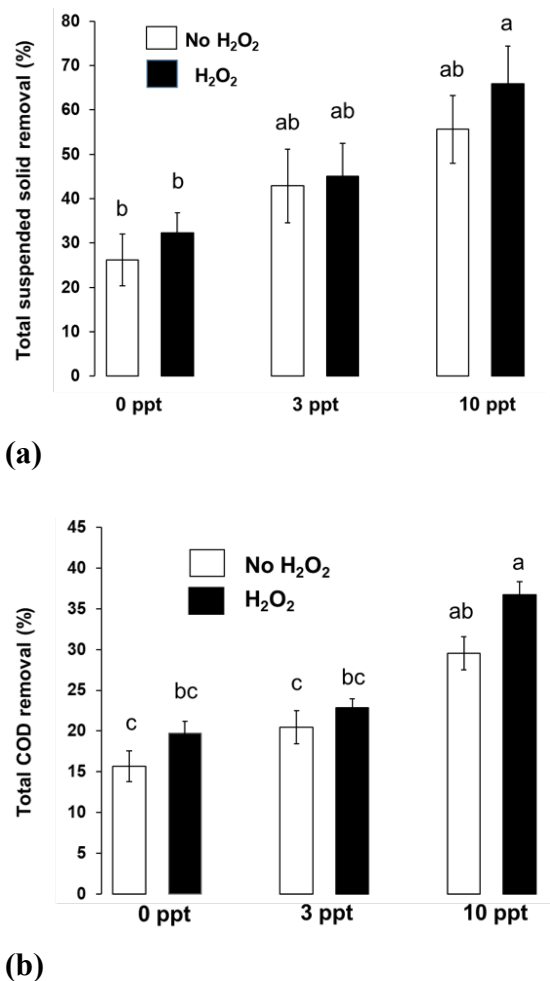


FIGURE 6.4.2. Removal efficiency of organic matter from the water as foamate after 6 hours in the six treatments with FF as percentage removal of a) TSS and b) total COD. Treatments include salinity in three levels (0, 3, or 10 ppt) and the presence (10 ppm H₂O₂) or absence of hydrogen peroxide (no H₂O₂). The data presented is mean \pm SE of three replicates, and the different letter denotes significant differences between groups.

TABLE 6.4.1: Range of selected RAS water quality parameters used for the batch experiments over three days prior to the treatments.

Variables	Range
Temperature	15-16.5
pH	7.4-7.8
Oxygen conc. (mg O ₂ l ⁻¹)	>9
Bacterial Activity (K (h ⁻¹))	0.16- 0.47
Particle numbers (N ml ⁻¹)	1.21-1.70 · 10 ⁵
Turbidity (NTU)	4.4-5.8

6.4.2 Experiment 2 - Foam fractionation and Ozone

No significant fish mortality or differences in growth or feed conversion rates were observed during the 8-week trials investigating the effects of foam fractionation and ozone on water quality (data not shown). Oxygen saturation ranged between 85 and 90 %, pH between 7.0 and 7.3, and temperature between 17 and 21°C throughout the trial. There were no differences in ammonium and nitrate levels by the end of the trial, while nitrite was significantly lower in systems fitted with foam fractionators (Tab. 6.4.2).

Particles: Systems treated with ozone displayed rapid (> 80%) decline in microparticle numbers within the first week maintaining a low level of particles until the end of the trial. Systems fitted with foam fractionators showed a slower reduction in particle numbers, resulting in a final reduction of 58 % compared to the control. By the end of the trial, both the use of O₃ and FF had led to significant reductions in particle volume. Also particle surface area was affected by the two treatments: Foam fractionation resulted in a 53 % reduction of total surface area, O₃ treatment in a 68 % reduction and a combination of both treatments in an 83 % reduction of particle surface area compared to the control. β values were only affected by the use of ozone. Control systems and systems with foam fractionators had similar β values by the end of the trial (3.74 and 3.77, respectively), while systems treated with O₃ displayed significantly lower β values (3.17 and 3.24 for O₃ and FF+O₃ treatments, respectively).

Microbial activity: Bacterial activity, measured both with the H₂O₂ degradation rate assay and BactiQuant, declined rapidly in systems treated with ozone, with activity after one week being reduced by 91 % in systems with ozonisers only and 96 % in systems with FF+O₃ treatments compared to the control (H₂O₂ degradation rate assay). However, only systems where ozonisers and foam fractionators were combined were able to maintain this low bacterial activity (90 % reduction) throughout the trial, whereas in the systems with only ozone the microbial activity fluctuated after the first week of the trial.

Turbidity and UVT: Turbidity was reduced and UVT increased by both foam fractionation and ozonation (Tab. 3.4.2). By the end of the trial, a 65 % reduction in turbidity and a 15 % improvement in UVT was achieved by foam fractionation, a 38 % reduction in turbidity and a 43% improvement in UVT by ozonation and a 79 % reduction in turbidity and 47% improvement in UVT by combining both treatments. However, similar to microbial activity, turbidity appeared to increase after an initial drop when applying ozone alone.

BOD₅, COD: Total BOD₅ was significantly affected by both foam fractionation and ozonation resulting in reductions of 51 %, 43 % and 75 % for FF, O₃ and FF+O₃, respectively, compared to the control. The development in BOD_{5-Part} was similar to BOD_{5-Tot} for all treatment combinations (Tab. 6.4.2). By the end of the trial, foam fractionation alone and direct ozonation had led to similar reductions in BOD_{5-Part} compared to control of 56 and 54 %, respectively, while a combination of the two resulted in an 84 % reduction. In contrast to total and particulate BOD₅, the different treatments seemed to have little effect on BOD_{5-Diss}, (Tab. 6.4.2). COD_{Total} in the last 3 weeks was significantly affected by both foam fractionation and ozonation with a combination of the two resulting in the largest decrease compared to the control (58 % reduction). Foam fractionation and ozonation by themselves resulted in similar reductions of 39 and 33 %, respectively. Both treatment types affected COD_{Part}, with reductions of 69, 36 and 80%, respectively, in systems with foam fractionation, ozonation or a combination of the two (Tab. 6.4.2). Dissolved COD was also significantly affected by the different treatments. As with every other metric, the combination of foam fractionation and ozonation had the largest effect reducing COD_{Diss} by 40 %. Foam fractionation by itself reduced COD_{Diss} by 16 % while ozonation reduced it by 31 %.

Total COD in biofilters: Although all treatments seemingly lowered COD_{Tot} levels compared to the control, there were no significant differences by the end of the trial in total COD in the biofilters, although the total COD in biofilters was approximately 17 % lower value in systems with ozonation only, and 23 % lower in systems with foam fractionation.

TABLE 6.4.2. Particle concentration and size, turbidity, UVT, microbial activity, biological and chemical oxygen demand and nutrient concentrations in the water and chemical oxygen demand in the biofilter during 3 last weeks of sampling (mean \pm SD). (*) Indicates statistical significant effects of the main factors (FF and O₃), while (°) indicates interactions between main factors.

<i>Treatment</i>	<i>Control</i>	<i>Foam fractionator</i>	<i>Ozone</i>	<i>Foam fractionator + Ozone</i>	<i>Units</i>
Num. Particles	2.43 \pm 1.38	1.01 \pm 1.01*	0.42 \pm 0.22*	0.27 \pm 0.14	million ml ⁻¹
Vol. Particles	0.037 \pm 0.012	0.014 \pm 0.003*	0.025 \pm 0.006*	0.009 \pm 0.002	mm ³ ml ⁻¹
S. A. particles	30.39 \pm 8.77	14.32 \pm 5.75*	9.84 \pm 2.52*	5.23 \pm 1.95	mm ² ml ⁻¹
β value	3.74 \pm 0.24	3.77 \pm 0.28	3.20 \pm 0.22*	3.28 \pm 0.26	dimension-
Turbidity	7.02 \pm 2.56	2.46 \pm 0.83*	4.34 \pm 1.07*	1.49 \pm 0.43	NTU
UVT	51.72 \pm 2.59	59.37 \pm 2.01 ^a	73.75 \pm 4.48 ^a	75.94 \pm 1.36	% trans-
H ₂ O ₂	0.84 \pm 0.24	0.33 \pm 0.17*	0.44 \pm 0.27*	0.08 \pm 0.03	k ⁻¹
Bactiquant	77011 \pm 32480	35779 \pm 24185	65674 \pm 30563	17110 \pm 6172	BQV
BOD _{5Total}	6.09 \pm 1.05	2.99 \pm 0.89*	3.45 \pm 0.55*	1.53 \pm 0.24	mg O ₂ l ⁻¹
BOD _{5Dissol}	0.82 \pm 0.13	0.67 \pm 0.10	1.01 \pm 0.33	0.67 \pm 0.04	mg l ⁻¹
BOD _{5Part}	5.27 \pm 0.98	2.33 \pm 0.88*	2.44 \pm 0.69*	0.86 \pm 0.023	mg l ⁻¹
COD _{Total}	37.64 \pm 5.86	22.84 \pm 2.70*	25.21 \pm 2.90*	16.01 \pm 1.49	mg l ⁻¹
COD _{Dissol}	21.36 \pm 1.71	17.84 \pm 1.01*	14.83 \pm 1.05*	12.78 \pm 0.78	mg l ⁻¹
COD _{Part}	16.29 \pm 4.74	5.00 \pm 2.91*	10.39 \pm 2.93*	3.23 \pm 1.94	mg l ⁻¹
Ammonium	74.7 \pm 30.0	83.8 \pm 17.9	88.5 \pm 36.7	82.9 \pm 11.6	μ g NH ₄ -N l ⁻¹
Nitrite	119.3 \pm 24.5	77.5 \pm 20.6*	104.0 \pm 24.3	70.5 \pm 24.26	μ g NO ₂ -N l ⁻¹
Nitrate	57.5 \pm 2.57	56.7 \pm 2.70	57.4 \pm 2.33	56.6 \pm 2.65	mg NO ₃ -N
Biofilter COD	9.3 \pm 2.2	7.2 \pm 2.4	7.5 \pm 1.9	7.2 \pm 1.0	g



FIGURE 6.4.3. Effect of the different treatments on water clarity. From left to right: Ozone, Ozone + foam fractionator, control and finally foam fractionator.

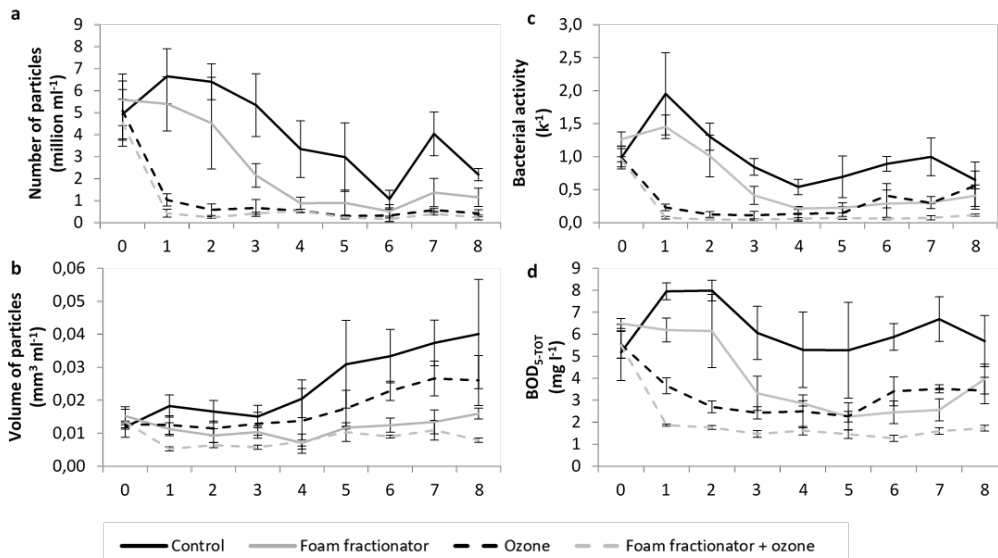


Fig. 3.4.4. A) Changes in numbers of particles (million mL⁻¹), B) volume of particles (mm³ mL⁻¹), C) Bacteria activity (k⁻¹) and D) BOD_{5-tot} (mg L⁻¹) during the eight week trials (mean ± SD). (Black solid line) control, (grey solid line) foam fractionator, (black dotted line) ozone, (grey dotted line) combination of foam fractionator and ozone.

Our results document the potential of FF in improving the water quality, meeting an increasing need from aquaculture industry to find viable solutions to control the accumulation of fine solids and bacteria in RAS. The pilot scale RAS study with FF and ozone also demonstrated a reduction of organic matter build-up inside biofilters. This is a clear advantage, since it will improve the performance of biofilters and facilitate the management of the RAS unit. The documented effect of FF in freshwater is similar to what can be achieved in seawater (where FF is applied), which has not been demonstrated before. The effect of FF can further be strengthened by combination with salt, hydrogen peroxide and ozone. Our results therefore suggest FF as a new alternative solution to improve water quality that can be directly applied and further optimized. The existing solution to deal with bacterial build-ups in RAS is primarily by use of disinfectants. While chemical disinfectants (i.e. formaldehyde, hydrogen peroxide and peroxy-acetic acid) are effective at reducing or controlling bacterial numbers in the water, these compounds fail to address the basic cause – organic matter build-up. Even though the disinfectants can reduce or eliminate bacteria and parasite when properly applied, they do not as such reduce the organic matter in the system, but rather add substrate and can potentially effect the biofilter. In contrast, ozone and hydrogen peroxide do not add carbonaceous compounds and are neutral in a mass balance perspective as opposed to formalin and peracetic acid.

6.5 Survey

Main results:

- Most common motivation for adapting new technology is optimization of for instance, processes, space or water quality
- Producers adapt new solutions regularly, mainly based on equipment that is already present in the farm
- Producers are most likely to adapt a new technology after having seen it used in practise
- Biological control as a solution to water quality problems is not considered relevant

6.5.1 European scale

The willingness of European aquaculture farmers to participate in online surveys was minimal, yielding only seven responses. Among these, one respondent was involved with aquaculture production in Central America, one with aquarium (animal) production and one did not answer the majority of questions. In addition, four respondents had a background in Danish aquaculture.

In general, their answers reflected the produced species, of which three were bivalves (2 mussels, 1 oyster), and one warm water marine species (*Seriola lalandi*). Unfortunately these species are not representative for the overall production of aquatic species in Denmark, nor the production systems which TOBIFREE focus on. For this reason and the overall low number of respondents, the results from the online survey were not considered relevant for the project.

6.5.2 Danish scale

All seven contacted farmers were willing to participate in the survey, and one even emphasised a strong interest in providing further input to MST, with regard to particular topics outside the scope of the survey. Most of respondents farmed trout in freshwater ponds, although not all farmers covered the full life cycle of the animals. The farmers told that they adopt new solutions regularly but the degrees varies across the production chain. Most changes are related to management of water and its quality, whereas larger changes, such as the introduction of RAS, happen less often. Full answers can be seen in App. 7.

6.5.2.1. Experiences with a recent uptake of a solution

Information transfer regarding recent solutions which have been adopted: Producers learn about solutions primarily from colleagues or alternatively equipment suppliers or researchers. Some also just try things out by themselves. Producers trust new solutions when they have seen them demonstrated in action, e.g., at other producers or following their own pilot tests in small scale. In contrast, training was not generally needed for the adopted solutions.

Motivation for adapting new technology: The motivation to test new solutions will depend on the particular challenge such as e.g., moving larger fish, creating better work environment or improving fish welfare. Thus there is no single motivation for testing new things. However, 'optimization' is the overarching goal of most activities and relative advantages achieved by the new solutions related to e.g., more effective use of resources such as space (within their permit), adding of oxygen, but also more efficient use of labour can be relevant. Concerning the trialability of the new solutions, most new solutions were based on equipment which was already present at the farm, meaning the producers were familiar with the functionality, but adopted new practices or adjusted usage.

Economic factors which were relevant for the adoption of new solutions: The primary costs related to the adopted solutions were for several of the producers primarily related to the effort (working hours) for setting up and testing, although some farmers also found buying new products and services as the primary cost-driver.

6.5.2.2. Future challenges and solutions

Removal of off-flavour is a key R&I challenge for Danish freshwater trout farming, especially in RAS, which several farmers do not believe can provide optimal conditions for fish, both in terms of welfare and quality of the final product. Other challenges which should be addressed through research, innovation and development relates to e.g., food conversion, survival and disease management such as vaccines. The challenges for adopting more RAS among farmers are diverse, including lack of systems to clean water, regulate temperature and remove pathogens, as well as the cost. Almost none of the producers had heard of zooplankton as an example of biological control in RAS, but also did not think it was relevant to them as they did not see it as a solution in freshwater systems.

7. RECOMMENDATIONS AND FUTURE PERSPECTIVES

Biofilter: Stable and optimal biofiltration is of outmost importance in line with the associated management. This biologically driven treatment unit is much more complex than often assumed and gives rise to much consideration during the design and operation phases. Surprisingly few studies have investigated microbial dynamic, trophic interactions and mass balances related to operation conditions, although – as shown by our experiments – biofilter communities can have high turnover rates and predator-prey interactions that might keep the potentially harmful micro-organisms under control. Some of the important and fundamental parameters such as biofilter type (fixed vs. moving bed), hydraulic and retention time, feed loading and back washing events needs more attention, as well as the consequences of water treatment practices (disinfection, solids removal, foam fractionation etc.) on the performance of biofilters. These studies have to be performed under controlled conditions and eventually verified under commercial conditions.

Biological control: The two options for biological control of harmful micro-organisms in RAS could be zooplankton, such as cladocerans, or organisms living on biofilter, such as ostracods. Both cladocerans and ostracods can have high clearance rates, and thus capacity to reduce the concentrations of micro-organisms. Also, our results confirm the earlier findings on the ability of many cladocerans to feed on toxic algae, and suggest that species from lakes containing toxic algae (such as cyanobacteria) are efficient in feeding on these. However, the technological challenges in installing a ‘cladoceran filter’ are likely to be large and remain unexplored. In contrast, monitoring and eventually adjusting the concentrations of organisms that are already on the biofilter could be more feasible, and ostracods might be a good candidate for such organisms. This needs a better understanding of the predator-prey dynamics on biofilters and the effect of e.g., maintenance practises on them.

Physical control: The potential of FF deserves further attention as it is currently the most convincing and economically feasible way to address the challenges related to water quality in RAS. There is still a need to evaluate the processes at a larger commercial scale, as well as to work on methods to improve FF efficiency. Likewise, understanding the natural cycles and mass balances of organic matter within the RAS loop, especially concerning the storage and release of organic matter from biofilters, is essential. This knowledge is needed to optimize treatment processes of FF and biofilters, including management practices, which will lead to more safe and stable RAS with improved rearing conditions, reduced costs and reduced use of biocides.

The results from the two trials with FF demonstrate that fine solids (bacteria and micro particles) can be trapped and hence taken out of the water. This removal of particulate organic matter from the water phase represents a new water treatment option for RAS. It potentially opens up for a more sustainable and proactive management practice focusing on bringing down bioavailable organic matter which so far has been very difficult to handle. FF will thereby lower the carrying capacity of the system with direct implications of reducing the bacterial load. The positive effects include reduced microbial growth in the water phase – which will reduce the need to use chemical agents. Furthermore, increased and more efficient removal of organic matter in RAS will also reduce the load on biofilters, reducing uncontrolled growth and risks of unwanted events.

We foresee that FF can become a viable treatment option in certain freshwater RAS. Our studies show that current water treatment practice with hydrogen peroxide or salt can enhance the efficiency of the FF which further stresses this as an alternative to the current use of formalin and peracetic acid.

Promotion of new treatment methods: The perceived risk in engaging with new methods or technologies leads to slow adoption, and measures that could lead to minimising risk of negative impact on the production should therefore be advantageous. In order for a producer to consider testing a new solution they would likely prefer to observe it in action first. The new solution could be demonstrated by inviting the producers to research facility, and preferably involve the producers in the process early-on. The demonstration should be followed up with information about running costs (e.g., electricity consumption, maintenance needs).

Also results of experiences or trials with the solution should be easily available. If a producer has been involved in the trial, the report should also include an interview/video/material where the involved producers explain their experiences transparently, including both the positive and the negative aspects. These could be distributed to other producers and/or used to obtain further funding for later product development stages.

We propose the following when promoting the use of new treatment methods or other new solutions among Danish aquaculture farmers:

- 1) With regard to new treatments ozone is a well-known approach while zooplankton is not. This means that significantly more information and documentation would be needed to convince farmers to adopt such a treatment.
- 2) Ensure that it is possible to visit and observe sites where new solutions are being used, i.e. at an actual farm or dedicated test site.
- 3) Make sure that industry partners are willing to demonstrate the solution in action and share results, also with their competitors. Based on the interviews this should not be a problem in a Danish context due to generally good relationships among farmers and a joint understanding of the shared challenges and needs for solutions.
- 4) A third-party could serve as a facilitator in organising activities focusing on sharing knowledge and providing hands-on experiences.

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Appendixes

- Appendix 1.** The potential barriers for aquaculture farmers to adopt new technology cover both economic, political, social and policy issues, according to Kumar et al. (2018)
- Appendix 2.** Questions for the semi-structured interview of Danish aquaculture farmers
- Appendix 3.** Abundances of organisms in the different types of filter media and in different systems
- Appendix 4.** Abundance of organisms in the water surrounding the biofilter elements as a function of the time from backwash in facility 1.
- Appendix 5.** Abundance of organisms in the water surrounding the biofilter elements as a function of the time from backwash in facility 2.
- Appendix 6.** Answers to the surveys

Appendix 1. Factors driving the intensity and extent of technology uptake in aquaculture (based on Kumar et al. 2018)

Factor	Sub-category	Challenges	Mechanisms and factors which increase uptake
Information transfer	Media: Exposure to initial information about potential in technology is prerequisite for adoption, and can happen through both formal (e.g. dedicated journals) and informal sources (e.g. social media)	<ul style="list-style-type: none"> • The internet incl. social media, while a general advantage for knowledge seeking, do not necessarily make it easy to identify reliable and relevant technology information, nor is it necessary communicated at the right level 	<ul style="list-style-type: none"> • Non presented
	Knowledge extension: Dissemination of information and skills to end-users is needed to facilitate efficient uptake of technology in the industry	<ul style="list-style-type: none"> • Ability to transfer research outputs to impact at farm level • Technology complexity • Inability to consider local knowledge • Stakeholders whit out-dated knowledge 	<ul style="list-style-type: none"> • Extension personnel/agencies, can, due to their understanding of several contexts (i.e. practitioners and researchers or firms), bridge the gap between stakeholders. Extension work can be supported by e.g. system approaches where all actors from policy makers to technology developers learn from farmers to ensure information is transferred both back and forth. This can also be integrated in participatory research programmes.
	Training: In particular the improvement of technical knowledge and skills necessary to understand and use technology	<ul style="list-style-type: none"> • Information presented in training sessions must be very audience specific, and include topics related to e.g. risks and benefits • When experiences with a technology is little, there is an increasing risk that attitudes will be negative or technology disadopted 	<ul style="list-style-type: none"> • Training programmes at regional or national levels have been tested with success in several parts of the world. These can be both technical or hands-on, short or long-term • Community-based aquaculture have also been tested with in some developing countries • Training have also been found to support general innovativeness in some areas

Characteristics of the technology¹	Relative advantage: The perceived ability of a new technology to be comparatively better than its present alternative in terms of e.g. productivity, profitability, reduce risks etc.	<ul style="list-style-type: none"> • Drops in productivity can occur when adopting new technologies. This can be caused by e.g. insufficient knowledge and skills, costs related to the learning phase, organisational restructuring or demand for alternative investments • Farmers perception of risk and differences in risk-averse behaviour 	<ul style="list-style-type: none"> • Technologies with lower initial costs, are more likely to be adopted than those with high. This potentially explain why e.g. aquaponics, RAS and offshore systems often isn't exploited more. • Additional positive characteristics related to adoption are e.g. ability to: 1) reduce variation in costs; 2) reduce risks of using the technology itself.
	Compatibility: The ability of new technology to meet the requirements incl. past experience of likely adopters	<ul style="list-style-type: none"> • Compatibility refers to both the social, local and ecological context and which all must align • Public concern for e.g. increased pollution or GMO are examples of challenges, as adoption of the technology could come with a public opportunity cost or risk 	<ul style="list-style-type: none"> • Non presented
	Complexity: In particular the perceived challenge related to understanding and using the technology	<ul style="list-style-type: none"> • Adoption is negatively affected by the users perceived impression of the technology's complexity, e.g. extent and degree of change needed from present technology or management approach 	<ul style="list-style-type: none"> • Approaches to reduce the perceived complexity of a technology should benefit adoption • Significant improvements to key business parameters (e.g. labour) enhance uptake despite complexity
	Trialability and observability: The ability to try, test and familiarise with the new technology	<ul style="list-style-type: none"> • Lack of ability to observe and test innovations or technology can reduce the likeliness of adoptions 	<ul style="list-style-type: none"> • Adoption is likely to happen more often or quickly if the technology can be tested in a relevant context • 'Visible' innovations appear to be more easily adopted likely due to the better ability to demonstrate key selling points including e.g. improved output. Demonstration sites or ability to test in small scale at own farm are examples of this.
	Divisibility: The ability of a technology to be used at different scales or to a limited degree	<ul style="list-style-type: none"> • Innovations and technology packages which are tightly bundled with little flexibility concerning use, initial cost etc. can reduce chance of uptake 	<ul style="list-style-type: none"> • The ability to adopt parts of an innovation or technology (i.e. at lower initial cost) can facilitate uptake • The ability to modify parts at farm level are potentially important. It supports alternative uses of the innovation and the ability to substitute parts with better or cheaper components
	Complementarity: The ability of a technology to support adoption of another technology or input	<ul style="list-style-type: none"> • Non mentioned 	<ul style="list-style-type: none"> • Existence of complementary systems or technology likely facilitate uptake of new
Economic factors²	Profitability: In particular the expectation of increased profitability	<ul style="list-style-type: none"> • Non mentioned 	<ul style="list-style-type: none"> • Greater anticipated profitability is likely one of the clearest determinants for adoption of new technology or aquaculture approaches
	Input and output prices: In particular prices on final products or key inputs such as feed	<ul style="list-style-type: none"> • Changes in input and output prices directly affect the relative profitability of adopting a technology 	<ul style="list-style-type: none"> • Technology adoption is likely to be positively affected where it e.g. can increase the efficient use of input when these have a high price

		<ul style="list-style-type: none"> • The choice between e.g. productivity enhancing solutions in contrast to risk reducing technologies can be influenced by input and output prices • Volatile prices can make farmers delay decisions related to adoption of technology • High prices on e.g. feed can lead to suboptimal feeding strategies • Technology facilitated increases in production, i.e. supply (output), can end up reducing the prices for end product 	
	Availability of capital:	<ul style="list-style-type: none"> • Lack of capital reduces farmers ability to invest in new technology • The risk perception of the industry can be a challenge in relation to investors • Availability of capital is particularly relevant when adopting technology with a higher initial cost 	<ul style="list-style-type: none"> • Funding for aquaculture development can in some cases be supplied by alternative sources. These other funding lines including local governments, the World Bank or similar political development programmes
	Labor availability: In particular in relation to seasonality and skill	<ul style="list-style-type: none"> • Changes in labour availability can strongly influence the ability to adopt new technology • Labour wages or scarcity can increase the demand for labour saving technology, just as labour intensive technologies can have the opposite impact • Labour aspect also relate to a technology's impact on the ability of workers and managers to have leisure time. Labour intensive technology can thus reduce willingness to adopt 	<ul style="list-style-type: none"> • Farmers are only likely to adopt new technology if sufficiently skilled labour can be employed • Technology with lower demand for labour or management are more likely to be adopted, when labor is sparse and/or when wages are high.
Farm characteristics	Farm size:	<ul style="list-style-type: none"> • Scale dependent technologies, e.g. indivisible technologies are less likely to be adopted by smaller farmers • Improvements in cost efficiencies related to scale are less likely to be possible in smaller farms • Financial flexibility and resilience is likely lower in smaller farms 	<ul style="list-style-type: none"> • Adoption of new technology is in general more likely to happen in larger farms, though smaller farms are likely to catch up later

	Ownership and tenure:	<ul style="list-style-type: none"> Land owners can hold significant power over the farmers, as they influence contract conditions (i.e. long or short term) including credit opportunities 	<ul style="list-style-type: none"> Long term contracts or owner operated farms might positively influence adoption of technology (Mainly based on studies from poor countries)
Sociodemographic and institutional factors	Age:	<ul style="list-style-type: none"> It is not generally clear whether age is an important determinant of technology adoption in aquaculture, though higher age in other areas can influence adoption negatively 	<ul style="list-style-type: none"> While the level of experience with aquaculture systems potentially facilitate technology adoption, this is also tightly connected to age, leaving this determinant difficult to address
	Human capital: In particular	<ul style="list-style-type: none"> Lack of relevant human capital, often seen in smaller farms, is likely to cause less efficiency and negatively influence ability overcome the complexity related to adopting new technology 	<ul style="list-style-type: none"> Non presented³
	Location: Mainly as distance to other farmers or technology developers	<ul style="list-style-type: none"> Lack of proximity to other users and early adopters likely reduce uptake, i.e. for those outside established geographic clusters 	<ul style="list-style-type: none"> Companies, e.g. feed producers, can in some cases overcome the geographic challenge by facilitating information sharing between farmers in different regions
	Homogeneity: Particularly from the perspective of socioeconomics and culture	<ul style="list-style-type: none"> Knowledge on new technology are less likely to spread among farmers which face different socioeconomic contexts, i.e. large economic inequality, different languages etc. 	<ul style="list-style-type: none"> Before introduction of new technology it is relevant to understand the difference in socioeconomic contexts which face the relevant farmers In countries and regions with multiple ethnic groups this could include cultural differences, but probably not an issue in Denmark
	Policy interventions: Including financial and social programmes with particular incentives	<ul style="list-style-type: none"> Lack of relevant policies is generally a challenge for all sectors Subsidies can result in both “crowding out” private companies while in other cases the opposite 	<ul style="list-style-type: none"> Many different types of policy interventions can support technology adoption, from financial risk sharing to paid services for the producers

Factors influencing farmers capacity and willingness to take up new aquaculture technologies suggested by Kumar et al. (2018) drawing on results from both agri- and aquaculture. The factors described, builds on studies from studies across the world, and therefore cannot be taken as representative for all areas or societies. Similarly should it be noted that the listed aspects likely work synergistically.

¹With regard to the characteristics of the technology, Kumar et al. (2018) highlight, based on several studies, that personnel perceptions of the technology play a major role in the decision process. This is a challenge as these perceptions doesn't necessarily correspond to the available industry or academic knowledge. These considerations should be kept in mind for all of the technology aspects.

²Economic factors here refer to all financial aspects relevant to the aquaculture farm including anticipated financial benefits and risks etc.

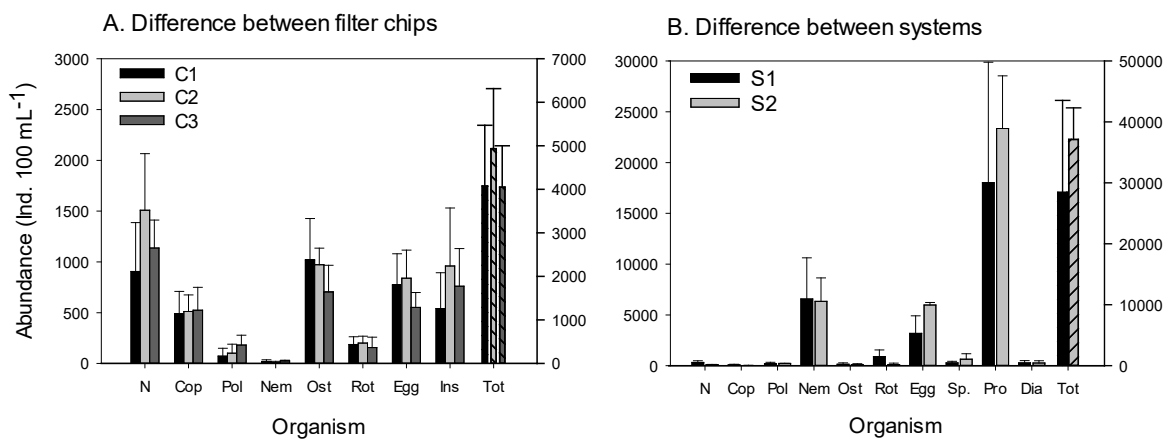
³The ability to overcome lack of human capital, is generally dealt with in other parts of the literature and therefore not covered here.

Appendix 2. Questions used in semi-structured interviews

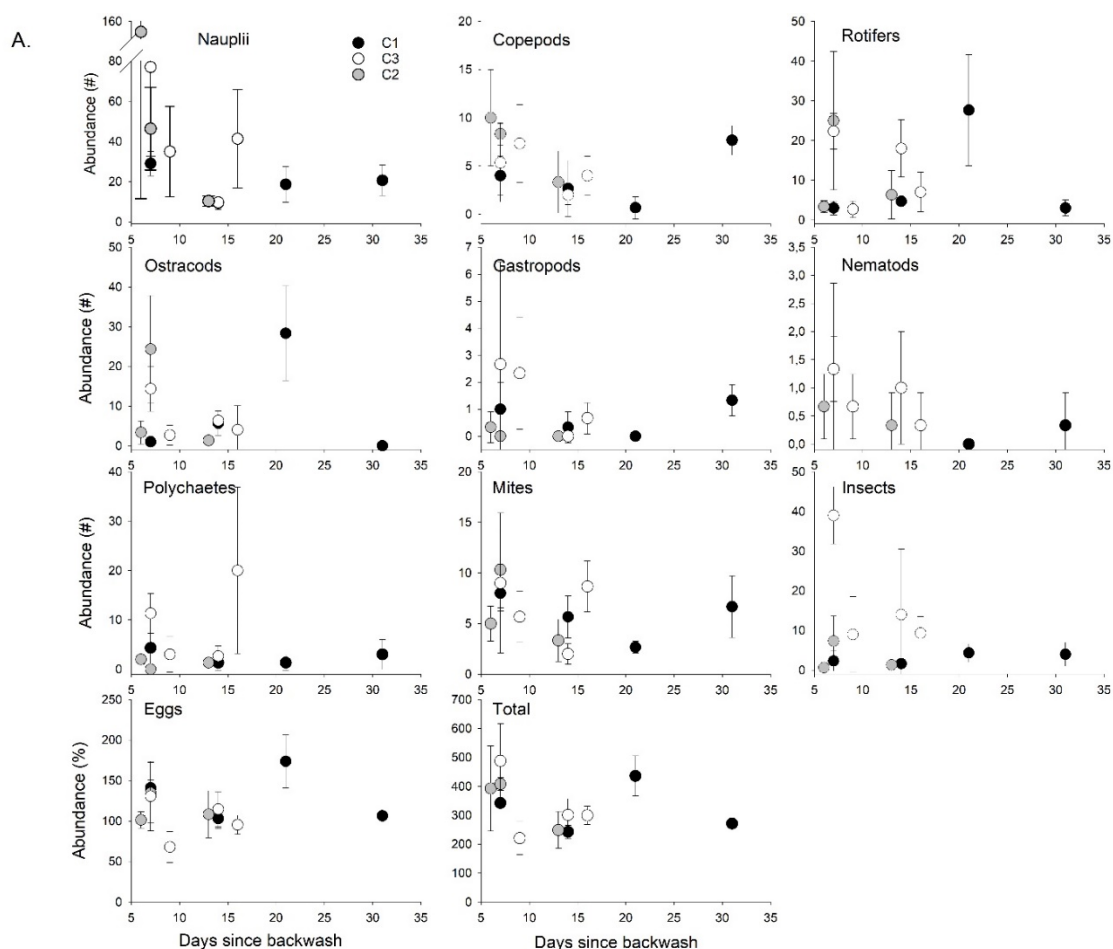
Erfaringer med implementering af ny teknologi og tilgange til arbejdet på faciliteten

1. Hvornår implementerede i sidst en ny metode eller løsninger til arbejdet på faciliteten
 - a. Hvad fik jer til at stole på potentialet i løsningen?
 - b. Hvilken teknologi eller løsning var det?
 - c. Hvor i jeres produktions-system indgik det?
2. Hvordan hørte du eller din virksomhed om denne mulighed?
3. Hvad motiverede jer til at teste det?
 - a. Hvad fik jer til at stole på potentialet i løsningen?
 - b. Havde det nogle relative fordele i forhold til jeres daværende løsning – og hvordan?
 - c. Havde i mulighed for at teste det inden i købte det?
 - d. Fik i nogen træning/oplæring i at anvende det?
4. Hvad var den primære udgift ved at teste den nye løsning?
5. Hvilken type information og validering bør være tilstede før at man som producent vil overveje at teste det?
6. Hvor i din egen produktion oplever du de største udfordringer, som man kunne håbe at kommende forskning og innovation kunne være med til at løse?
7. Hvis du ikke anvender RAS systemer i din virksomhed, hvad er så den største barriere for at i stigende grad anvende dette?
8. Er du bekendt med mulighederne for at bruge biologisk kontrol (zooplankton) til at nedbringe forekomsten af giftige alger i RAS?
9. Hvilken produktionstype har i (art, facilitet, livsstadier)?

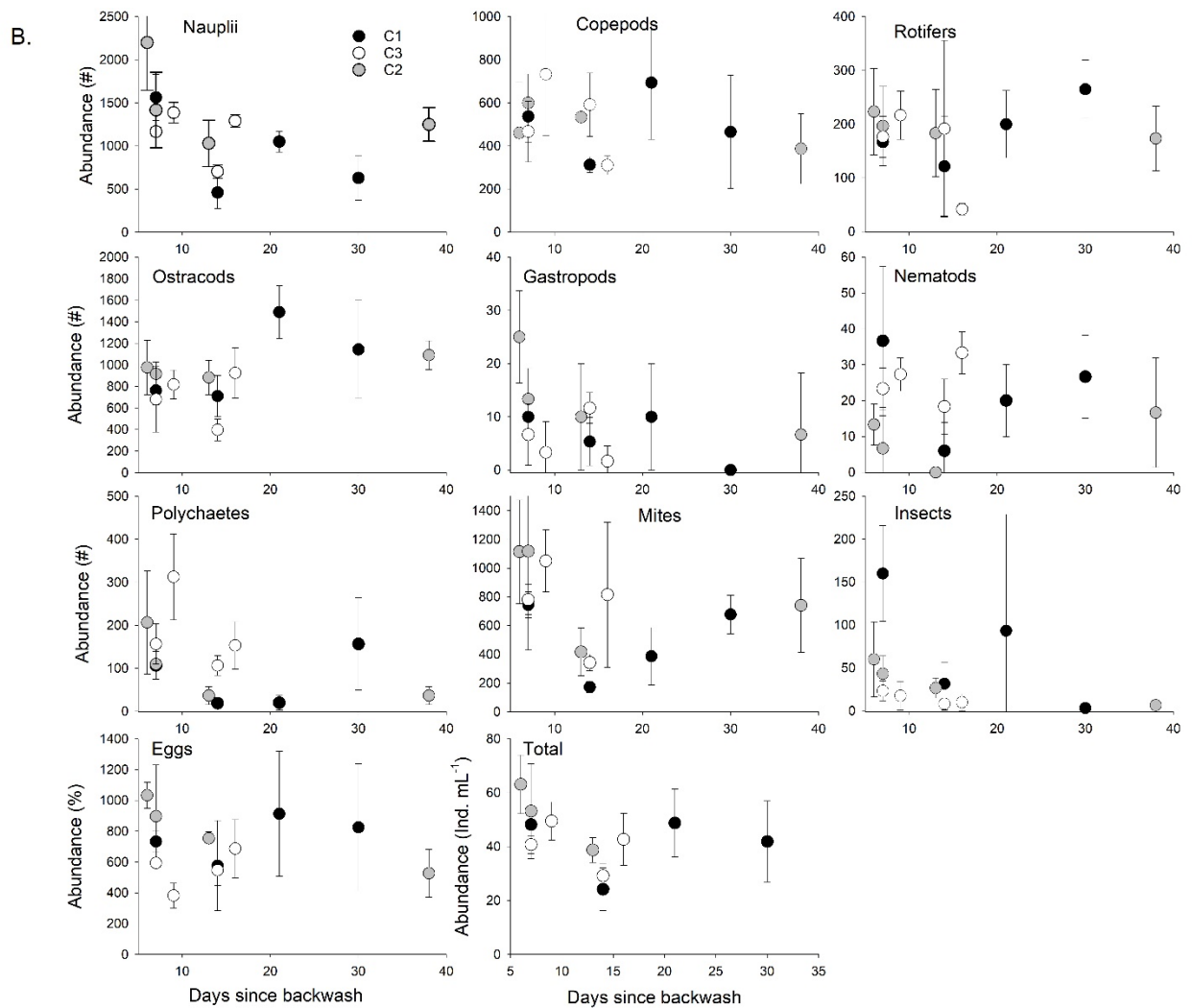
Appendix 3. Abundance (ind. 100 mL⁻¹) of protozoans, zooplankton and invertebrates on different types of biofilter media in the facility 1 and in the two different systems of the facility 2 (mean ± SD). Abbreviations as in Fig. 3.1.1.



Appendix 4. Abundance of different organism groups in the water around the biofilter, as a function of days after backwash.



Appendix 5. Abundance of different organism groups in the filter chips, as a function of days after backwash.



Appendix 6. Questionnaire and results from the surveys

Introducing text: Aquaculture Producer Survey 2020

The European aquaculture industry is an important provider of food for the world's growing population. To support the industry's development the European Union and its states therefore continuously invest public money in research and innovation.

However, the industry's diversity in relation to location, company size, use of systems, employees, value chains etc. likely present a challenge, with regards to the ability to take up new knowledge and technical solutions.

To overcome this challenge researchers, environmental agencies and companies could benefit from a common platform of understanding of the different requirements or barriers which the industry and its employees experience in relation to new solutions or technology.

Yet, this type of information is not widely available which reduces the ability of public research and innovation projects to align with the requirements of the industry, and deliver relevant solutions and information.

This online survey aims at closing this knowledge gap with regards to aquaculture producers in Europe, to improve common understanding of needs and solutions.

We therefore hope you will use 10 minutes to fill out this questionnaire and forward it to colleagues both within and outside your own company. Your answers are completely anonymous.

If you have direct comments or questions for us to answer, please contact chrii@aqu.dtu.dk directly.

The team behind this survey thank you for your time and willingness to share your experiences. Technical University of Denmark, Institute for Aquatic Resources.

The project is funded by the Danish Ministry for Environment and Food.

Result: Aquaculture Producer Survey 202

1. Your experiences with implementing new technology or approaches at your facility (characteristics of technology + economic aspects)	
When was the last time (year) that your facility implemented a new technology or approach at your facility (e.g. new elements into a monitoring system, new biosecurity protocol, new software, etc.)?	<ul style="list-style-type: none"> • 2014 (1) • 2018 (1) • 2019 (2)
What type of new technology or solution was it? Choose from the options below	<ol style="list-style-type: none"> 1) New feed type 2) New antibiotic based measure 3) New prophylactic measure (e.g. use of vaccines) 4) New chemical disinfectants for preventive or curative disease control 5) New farm operations (1) 6) Monitoring system (e.g. new types of sensors, PLC, daily routine monitoring other) 7) Software (e.g. batch-tracking software, daily routine monitoring software, other) 8) New type of equipment (e.g. pumps, valves, oxygen cones, bio-elements (e.g. wood), sorting & grading equipment, vaccination equipment, protein skimmers, other) (3)

Please describe it in detail... including e.g. where in the system you placed it.	<ul style="list-style-type: none"> • PP-Iltkasse fra Frea. Nursery. Bedre iltopløsning og som supplement til ilt dosering i de enkelte kar (Seriola lalandi 1-50 gram) • New flipping bag system for oyster farming • Start of mussel farming on Smartfarm systems • new machine for harvesting mussels placed it on the boat
How did you or other in your company learn about this solution or practice? (<i>more than one answer possible</i>)	<ol style="list-style-type: none"> 1) Other farms 2) Colleagues at own farm 3) Equipment supplier (1) 4) Research institution (1) 5) Consultant (2) 6) Current advisor 7) Other farms (1)
What motivated you or your company to test this new technology or solution?	<ol style="list-style-type: none"> 1) Part of funded project (1) 2) Curiosity driven (1) 3) Overall optimisation 4) Increase production capacity (3) 5) Energy reduction 6) Lower environmental impact 7) Increase safety/risk reduction (1) 8) Legal demand 9) Sudden die out of stock
What made you trust information about the benefits of the new solution (e.g. you knew others who already used it)?	<ul style="list-style-type: none"> • Networking and contact to producer • I saw videos • We had tested it • It was working in other companies
Did it have relative advantages compared to the present solution, and if yes, what? (<i>more than one answer possible</i>)	<ol style="list-style-type: none"> 1) Decrease costs (2) 2) Increase production (4) 3) Increase component performance 4) Legal compliance 5) Other
When you first heard about the technology, did you think it was ready to be implemented in the industry?	<ol style="list-style-type: none"> 1) Not ready 2) Ready for some applications (3) 3) Ready for most applications 4) Complete ready (1)
How easy did you think it would be to adopt (i.e. how challenging did you think it would be to understand and use it)?	<ol style="list-style-type: none"> 1) Very difficult 2) Difficult (1) 3) Neutral 4) Easy (3) 5) Very easy
Did you have an opportunity to test the equipment/solution, and if yes, - how did you test it? – in the entire production, a small portion (e.g. one out of several pens/tanks), a small pilot (e.g. in an experimental setup)?	<ul style="list-style-type: none"> • Pilot in few tanks first • Tested a small sample • Pilot test • I tried a machine a bit different from the one I made
Were you able to roll back to previous solution if it did not work to you or the company satisfaction?	<ul style="list-style-type: none"> • Yes • Yes • Yes • Yes
Did you receive any training/assistance in implementing the technology/change of operation/solution or did you do it on your own through information-searches and trial-and-error methods?	<ul style="list-style-type: none"> • Yes • No • Assistance • No
What was the main costs associated to the testing (buying or renting equipment, working hours etc.)	<ul style="list-style-type: none"> • No • Buying equipment • Working hours • I tried the machine in a project so its cost some work hours
2. Getting information about new technology and solutions in general (information transfer)	

Where do you normally seek information about innovative new solutions or technology (e.g. attend conferences, read printed aquaculture related journals, specific websites, learn from peers, consultants, suppliers, etc.)?	<ul style="list-style-type: none"> • Network • YouTube • Journals, consultants, suppliers, public research • From colleagues and suppliers
What do you normally consider the main barrier for you or your company, when it comes to adopting new technology or solutions? (more than one answer possible)	<ol style="list-style-type: none"> 1) Financial ability to invest (1) 2) Willingness to take risks (1) 3) Uncertainty about effects (2) 4) Restrictions on analytical verification 5) Lack of employee capacity to implement (1) 6) Too little time 7) Lack of interest from owner 8) Other (1)
What type of information and validation should accompany new solutions developed by public research and innovation projects, before you or your company would consider adopting it?	<ul style="list-style-type: none"> • Publications • YouTube videos of it in action • Impact, estimated production and costs • It should have been tested in a real production
Where in your production do you presently have the largest problems, which you believe research and innovation could help solve?	<ul style="list-style-type: none"> • Grow out • Permits, slow governmental agency • Immersion of smart farm units • The biggest problem is when you wanted to start something new its take a month/years for the government to give the permission,
If you do not have a RAS system in your company, what do you consider the main barriers for adopting recirculating aquaculture systems technology (e.g. location, access to water, discharge issues, license and approval periods, employees, investments etc.)?	<ul style="list-style-type: none"> • Have RAS • Cost and profitability
How familiar are you with the potential in using biological control (e.g. zooplankton) to decrease the risks of harmful algae blooms or N and P build up, in recirculating aquaculture systems?	<ol style="list-style-type: none"> 1) I have personal experience with this type of control 2) I am aware of the concept 3) I am unfamiliar with this approach (3) 4) Do not want to answer (1)
How familiar are you with the potential in using ozone as a water treatment approach in recirculating aquaculture systems?	<ol style="list-style-type: none"> 1) I have personal experience with this type of control (1) 2) I am aware of the concept (1) 3) I am unfamiliar with this approach 4) Do not want to answer (2)
3. The aquaculture facility you work at (optional to answer all questions)	
What country and region do you work in?	<ul style="list-style-type: none"> • Denmark • Denmark, Limfjord • Denmark, Limfjord • Denmark
What is the main purpose of your production? (more than one answer possible)	<ol style="list-style-type: none"> 1) Human consumption (4) 2) Release 3) Biocontrol 4) Aquariums 5) Feed (1)
What type of aquaculture system do you and your company work with? (more than one answer possible)*	<ol style="list-style-type: none"> 1) Photobioreactor (e.g. microalgae) (1) 2) Hatchery (1) 3) Larvae rearing (1) 4) Smolt production 5) Grow out (1) 6) Broodstock (1)
*Note – online one answered	
Where is the facility placed?	<ol style="list-style-type: none"> 1) Indoor

<i>(more than one answer possible)</i>	<ol style="list-style-type: none"> 2) Outdoor (1) 3) Land based (1) 4) Sea based (2)
What type of water system do you mainly use?	<ol style="list-style-type: none"> 1) Freshwater based 2) Marine water based (4)
What technical aquaculture system do you mainly use?	<ol style="list-style-type: none"> 1) Fully recirculated aquaculture system "RAS" (closed) (1) 2) Semi-circulated aquaculture system 3) Freshwater open flow-through system 4) Marine offshore (1) 5) Integrated multitrophic aquaculture (e.g. seaweed and fish) (1) 6) Do not want to answer (1)
What species do you farm?	<ul style="list-style-type: none"> • Seriola lalandi • Oysters • Mussels • Mussels
What life cycle stages of the produced species do your company work with?	<ol style="list-style-type: none"> 1) Eggs (hatchery) (1) <ul style="list-style-type: none"> • 1b) Gametophytes (macro algae) 2) Larvae (rearing) (2) 3) Fry 4) Fingerling (1) 5) Juvenile (2) 6) Adult (6) <ul style="list-style-type: none"> • 6b) Sporophyte (macro algae ready to harvest) 7) Spawning adult (broodstock) (1) <ul style="list-style-type: none"> • 7b) Reproductive macro algae
What types of biosecurity, prevention and veterinary options (strategies) do you apply to pest and disease control techniques and treatments do you use in relation to the farmed organisms?	<ol style="list-style-type: none"> 1) None (3) 2) Vaccines 3) Antibiotics 4) Biological control (e.g. cleaner fish) 5) Water disinfection with chemical therapeutics (e.g. formaldehyde, hydrogen peroxide, chloramine T, peracetic acid, etc.) (1) 6) UV 7) Ozone 8) Combination of UV + Ozone
How likely do you think it is that your company would be the first to test a new solution or technology?	<ol style="list-style-type: none"> 1) Very likely (2) 2) Likely (2) 3) Possible 4) Unlikely 5) Very unlikely
4. Background information about you (optional to answer all questions)	
How old are you?	<ol style="list-style-type: none"> 1) 10-17 2) 18-29 3) 30-39 (1) 4) 40-49 (1) 5) 50-59 (2) 6) 60-69 7) 70+
What is your sex?	<ol style="list-style-type: none"> 1) Male (4) 2) Female 3) Non binary 4) Do not want to answer
What is your highest achieved level of education?	<ol style="list-style-type: none"> 1) Primary school, secondary school, high school or similar (1) 2) Vocational education 3) College degree 4) Graduate degree (1) 5) Aquaculture degree (2)
What is your occupational degree in relation to aquaculture?	<ol style="list-style-type: none"> 1) Full time employed (2) 2) More than 50% employment 3) Less than 50% employment (2)
What is your job in relation to aquaculture?	<ol style="list-style-type: none"> 1) Aquaculture facility owner and manager (3) 2) Aquaculture facility manager (e.g. controls and coordinates work - employed by owner) (1) 3) Aquaculture technician (e.g. animal husbandry - employed by owner) 4) Aquaculture veterinarian

	<ol style="list-style-type: none"> 5) Aquaculture engineer (e.g. system optimization and maintenance) 6) Do not want to answer
Experience working within the aquaculture industry?	<ol style="list-style-type: none"> 1) Less than 5 years (1) 2) 5-10 years (1) 3) 10-20 years (2) 4) 21-30 years 5) More than 30 years 6) Do not want to answer
How important do you think it is for the industry to continuously test novel solutions or technologies in the production?	<ol style="list-style-type: none"> 1) Very important (2) 2) Important (2) 3) Moderately important 4) Slightly important 5) Unimportant
Would it be okay for you to be contacted by the research team for a follow up interview? If yes please provide contact information below (email and / or telephone number)	<ul style="list-style-type: none"> •

TABLE Y – Background information

For tables with no answers provided, these indicate the farmers reluctance to share that specific information. Each ‘answer number’ represent the same respondent throughout all the answers across the tables. Given the nature of semi structured interviews, the provided answers in the tables are the researchers condensation of key points raised by the respondents and thus not 100% copy of the provided oral answers.

Question	Answer
When did you last implement a new method or solution related to the work at the facility?	<ol style="list-style-type: none"> 1) Happens all the time. They just inaugurated to new pond systems. 2) Continuously 3) 3 years ago 4) March/april 2020 5) 14 days ago 6) 1989/99 7) Continuously over the last 6 months
What technology or solution was it?	<ol style="list-style-type: none"> 1) Two full pond systems 2) Automatic PH regulation (dosage mechanism) 3) Woodchip for water filtration (biofilter) 4) A pump solution to move larger fish instead of using a racket. 5) Cannot say – for business reasons 6) Introduction of RAS. They transferred a system to collect effluent like in pig production and made particular dams for this. 7) Adding of oxygen to central inflow of water
Where in your production system was it placed?	<ol style="list-style-type: none"> 1) – 2) Several parts where water needs right PH 3) Water filtration of effluent water 4) Last part 5) In connection with recirculation in model 3 system 6) Changing the water in the ponds 7) Inflow of water
Production type and species	<ol style="list-style-type: none"> 1) Trout Model pond and Pike perch (RAS) 2) Model 3 pond, buys eggs, otherwise produce trout up to around 550g 3) Trout, all life stages. 4) Model 3, trout, all life stages. 5) Model 1 +3, trout 6) Trout in ponds 7) Traditional trout ponds with all life stages. Consider it climate friendly as no electricity is needed (unlike RAS)

Categorised answers to semi-structured survey

Factor	Sub-category	Questions	Answers
Information transfer	Media: Exposure to initial information about potential in technology is prerequisite for adoption, and can happen through both formal (e.g. dedicated journals) and informal sources (e.g. social media)	<ul style="list-style-type: none"> Hvordan hørte du eller din virksomhed om denne mulighed? 	<ol style="list-style-type: none"> Colleagues, Equipment supplier and consultants in general It is a known technology, and we already have the dosage unit A DTU PhD student looked for a test site. They were curious and the student were very nice. Other farmers used it with success. Went out to see it. Emphasized that one is always welcome at each ours sites as they are good at working together to solve problems. Good collaboration between companies. When the need for new solutions arise, he and a colleague go out for “coffee” 2-3 places to hear more about their experiences. People are nicely honest and glad to tell what they would have done different also. Not two systems are identical. Tried things out them self Active in a society so knew the sector Own experiences and from colleagues – used FREA as the supplier
	Knowledge extension: Dissemination of information and skills to end-users is needed to facilitate efficient uptake of technology in the industry	<ul style="list-style-type: none"> Hvad fik jer til at stole på potentialet i løsningen? 	<ol style="list-style-type: none"> Believe the producer Had realised there was a problem which needed to be solved. The technology (from Oxyguard) was well known and they had partial experience dosage pumps for other things (hydrogen peroxide) Had made tests / demonstrations Saw that it worked other places Repeated tests. It work one place and then they scaled it up with success They tested and developed it them self Colleagues had good experiences (a certain platform for testing at FREA). And so far they have been satisfied though the solution does come with an operating cost.
		<ul style="list-style-type: none"> Hvilken type information og validering bør være til stede før man som producent vil overveje at teste det? 	<ol style="list-style-type: none"> Man skal enten se noget (og tror på det), man kan køre andre steder hen. Andre gange er det dem selv der tester (det skal kunne løse et problem effektivt, mindske omkostning eller øge produktion) All facilities are different, making it possible to test different things. This means there is not one solution as many parameters will depend on the facility. Electricity consumption, maintenance needs, longevity are generally important. They have as an example made tests with ocher-filtration.

			<p>3) Is on the board of organisations which work for the development of a test centre where all companies can come and see new solutions being demonstrated.</p> <p>4) Being able to see it work</p> <p>5) Answer will depend very much on who you ask. Some would look into the theoretical foundation to understand the problem/solution, others would go one step at a time and continuously check results. When looking for literature they use Google. They rarely finds something in Danish except for reports by Dansk Akvakultur often authored by researchers from DTU Aqua or KU plus particular vets or a guy from Aqua circle.</p> <p>They also collaborate with researchers from KU and DTU Aqua on e.g. vaccines testing of water samples etc.</p> <p>They also contact consultants in the sector e.g. from FREA.</p> <p>Similarly do they get advice on solutions from people in-house.</p> <p>However people can have many feelings involved in some of the challenges.</p> <p>6) Larger companies working with RAS related solutions often cannot document that it does not affect the quality of the fish. A problem, as large systems with RAS are very risky as the quality of the fish can quickly drop, with large economic impacts</p> <p>7) You would like to observe it, or potentially read reports about experiences with it</p>
	<p>Training: In particular the improvement of technical knowledge and skills necessary to understand and use technology</p>	<ul style="list-style-type: none"> Fik i nogen træning/oplæring i at anvende det? 	<p>1) –</p> <p>2) Originally there was a manual, and then they ran test at different scales</p> <p>3) Due to their curiosity regarding the ongoing (DTU phd) research project they learned a lot. Now they also have good contact with RUC master students who is developing plant lagoons, which their sheep now go out and eat.</p> <p>4) Got help to identify the right dimensions</p> <p>5) –</p> <p>6) No. They tested and developed them self</p> <p>7) No. But you order based on the desired flow. You can observe if you add too much (ånderør).</p>
<p>Characteristics of the technology¹</p>	<p><i>(This overall question is not in Kumar et al (2018) list</i></p>	<ul style="list-style-type: none"> Hvad motiverede jer til at teste det? 	<p>1) Generelt kan det være lovgivning, øget produktion – alt afhænger omstændighederne...</p> <p>2) Optimization of production, presently to many fish die, and too low welfare</p>

			<ol style="list-style-type: none"> 3) Curiosity regarding trying out new things, and obviously also interested in producing more within the permit 4) Had never had so large fish earlier, so something new had to happen to lower demand for labor. 5) Better work environment and use of time 6) Wanted a better way to produce considering both employees (work environment), environment and fish. 7) Optimization of the production from an environmental perspective which benefit the welfare of the fish
	<p>Relative advantage: The perceived ability of a new technology to be comparatively better than its present alternative in terms of e.g. productivity, profitability, reduce risks etc.</p>	<ul style="list-style-type: none"> • Havde det nogle relative fordele i forhold til jeres daværende løsning – og hvordan? 	<ol style="list-style-type: none"> 1) Had no examples 2) They had model 3 system, so stable parameters are wanted. This is challenging as the surrounding environment can change 3) Relatively cheap economic solution to discharge of nitrogen and phosphorus 4) Reduced demand for labour 5) – 6) Could produce more fish (in a better environment) in less space with added benefits for work environment. Productivity rose. The use of pure oxygen was particularly good. 7) Better welfare for the fish, better use of the oxygen due to better way of adding it.
	Compatibility:		
	Complexity:		
	<p>Trialability and observability: The ability to try, test and familiarise with the new technology</p>	<ul style="list-style-type: none"> • Havde i mulighed for at teste det inden i købte det? 	<ol style="list-style-type: none"> 1) Intet konkret eksempel ud over de to nybyggede anlæg. Fremhæver at man aldrig bygger helt ens i modsætning til landbruget, for dermed at kunne teste fordele og ulemper (læring). Der er også flere ”skoler” inden for akvakultur. 2) Had it already and used it 3) Had tested 4) Already had the pump, so had to rebuild it to it could fit both small and large fish 5) – 6) Tested and developed it them self 7) No. But could see it with colleagues (i.e. other farmers) + demonstrations at FREA. It is particularly important in Denmark, where there isn’t so many many farmers. Important to be able to observe in being used.
	Divisibility:		
	Complementarity:		

Economic factors²	Profitability: In particular the expectation of increased profitability	<ul style="list-style-type: none"> Hvad var den primære udgift ved at teste den nye løsning? 	<ol style="list-style-type: none"> Purchase and operation The hours for testing Setting up the solution (operation was neutral i.e. woodchip filtration) Presently building indoor production facilities due to predators (herons and raccoon dog), but still using woodchip filtration. Here the flush water from drum filters and “slam kegler” and filters are the largest challenge. Working hours Working hours for a subcontractor who was also responsible for the maintenance of x. The largest challenge was not economic but that the authorities did not understand the idea The purchase
	Input and output prices:		
	Availability of capital:		
	Labor availability:		
Farm characteristics	Farm size:		
	Ownership and tenure:		
Sociodemographic and institutional factors	Age:		
	Human capital: In particular		
	Location:		
	Homogeneity:		
	Policy interventions:		

TABLE Z – Forward looking

Question	Answer
Where in your production do you experience the greatest challenges, which research and innovation could help solve ?	<ol style="list-style-type: none"> Off-flavour in fish, nitrogen pollution (at their marine site), investment needs in ponds Cleaning of water (pathogen removal) due to high mortality and need to improve fish welfare. Also important with better feed conversion rates Locations to have aquaculture is not sufficient. Less and less areas and water gives less opportunities for development. Presently it is primarily popular for marine production which do not have space or can afford it.

	<ol style="list-style-type: none"> 4) The eggs are not in as good condition as they used to. The mother fish's conditions are worsening. It is a big challenge to keep both the small and large specimens alive. They are more susceptible to diseases. Believe it is related to the life cycle of the fish which is now unnatural due to the breeding regime which have enabled year round production of eggs. 5) Removal of off-flavour in fish produced in recycled water would be great. Presently lots of fresh water is just the best solution. So it would be great to solve this challenge. Additionally would a vaccine against BKD (bacterial kidney disease) be good as well as other vaccines in general 6) The challenge is that projects doesn't properly consider the full amount of factors which are needed to deliver good conditions for the fish. This impacts quality. The primary concern is to ensure that the fish gets what they need, so that the product quality does not drop. They recycle around 40% of water. They see a challenge in recycling more (even when adding oxygen), as it impacts quality, health and survival of fish. The present biofilters just are not good enough to ensure the necessary water quality. It is not good when e.g. environmental legislation indirectly reduces the quality of the products through demands for e.g. recirculation. Companies believe they can produce more than they actually can and they do not share information on some parameters. 7) Concerns regarding the nitrogen and phosphorus discharge (new regulation). It is not working, and find the challenging with the theoretical calculations which do not consider reality.
<p>If you do not use RAS in you company what is then the biggest barrier for using it to a larger extent?</p>	<ol style="list-style-type: none"> 1) Costs and lower quality of fish, particularly trout 2) The cleaning must improve. Presently the solutions are too expensive compared to the price they get for fish (the costs is unlikely to outweigh the benefit) 3) Is area-limited. The surrounding areas are "paragraph 3. Had tried without luck to offer an area 3 times larger in exchange for being able to develop on an area close to production facility presently paragraph 3. Want to be able to have a system which is 80% RAS, with the last life stages in non RAS to get rid of geosmin taste. Another place apparently does something like this. 4) Has a model 3 system with 8-110g fish in full RASS from groundwater. Larger fish gets water from the stream. Recycles as much as possible, but stream water temperature vary greatly and have periods with much ocher. Water gets too warm when recycling too much. 5) Permits from the municipality, and geosmin challenge. 6) That it cannot clean the water enough, thus only 40% recirculation 7) The economic cost related to energy consumption. There is obviously also an investment to be made, but that is okay as long as it brings a profit. Do not see big success with colleagues (other farms).
<p>Are you familiar with the potential in using biological control (zooplankton) to reduce the abundance of toxic algae in RAS?</p>	<ol style="list-style-type: none"> 1) No. Often not a problem in freshwater. 2) No. But have also never looked into it. Do not experience the problem. 3) No. Have heard of ozon. 4) No. but also do not have the problem. 5) No. Not a problem in freshwater RAS generally (algae have never been a problem at X) 6) No. But it is obviously always desirable to avoid chemicals. 7) Have heard about it but do not think it would be relevant

Additional comments

- 3) Would very much like a meeting with the ministry/agency about new ways to regulate the industry in a smarter and more efficient way
- 3) Those who provide loans for the sector has a bad impression of the sector which is a major problem.
- 6) Those who are supposed to help find financial support (tilskud), is unfortunately too theoretical and does not understand the actual conditions which the fish demand.

Towards biocide-free recirculating aquaculture systems

Recirculating aquaculture systems have been promoted as a sustainable supplement to net pen aquaculture and land-based flow-through systems. Recirculating aquaculture systems have numerous environmental assets such as decreased water consumption, but there are challenges related to water quality control and use of biocides in some systems.

Biofilters of the recirculating aquaculture systems harbored a rich community of protozoans and invertebrates such as copepods, ostracods, nematodes, polychaetes, rotifers and diverse eggs, and appeared to function as small ecosystems with active reproduction and predator-prey interactions and high turnover times. Dominating groups or species differed between the facilities, likely depending on salinity or light conditions, but were typically similar in the different systems at the same facility. Also, abundances of most organisms did not seem to change due to maintenance cycle, suggesting that the organisms resisted backwashing and remained in the system. Experiments investigating the interacting effects of propagule size, nutrient concentrations and the presence of a zooplankton (ostracod) suggested that ostracods that are naturally present on biofilters can control the abundances of microalgae, even at high nutrient concentrations. Similarly, diverse cladocerans had high feeding rates on microalgae, and particularly individuals that were collected from lakes with cyanobacteria blooms were able to feed on toxic cyanobacteria *Microcystis aeruginosa* at high rates.

Also, physical treatment methods were effective. Foam fractionation (FF) was a simple and effective water treatment technique to remove microparticles from freshwater recirculating aquaculture systems, and FF in combination with hydrogen peroxide and addition of salt led to significant reduction of both bacteria and turbidity. Pilot scale RAS trials documented beneficial properties of FF in terms of removal of microparticles, reduction of bacterial load, reduction of biodegradable organic matter and improvement of water clarity. FF combined with ozone led to an immediate and persistent improvement of water quality measured as bacterial load and microparticle concentrations. Both physical and biological treatment methods seem thus to be promising alternatives to chemical water treatment. Whereas biological treatments are still relatively far from application, physical treatment methods could become a viable option for freshwater recirculating aquaculture systems in near future.



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