ULTRASOUND DESINFECTION IN FRESHWATER AND MARINE RECIRCULATING SYSTEMS

Desislava Bögner*[1], Mirko Bögner[1], Kyra M. Böckmann[1], Kira Szidat[1] Anna Lenfers[1], Yara Zimmer[1], Christopher Behrendt[1], Marcus Salzwedel[1], Kerstin Klemm[1], Matthew J. Slater[1], Joachim Henjes[1]

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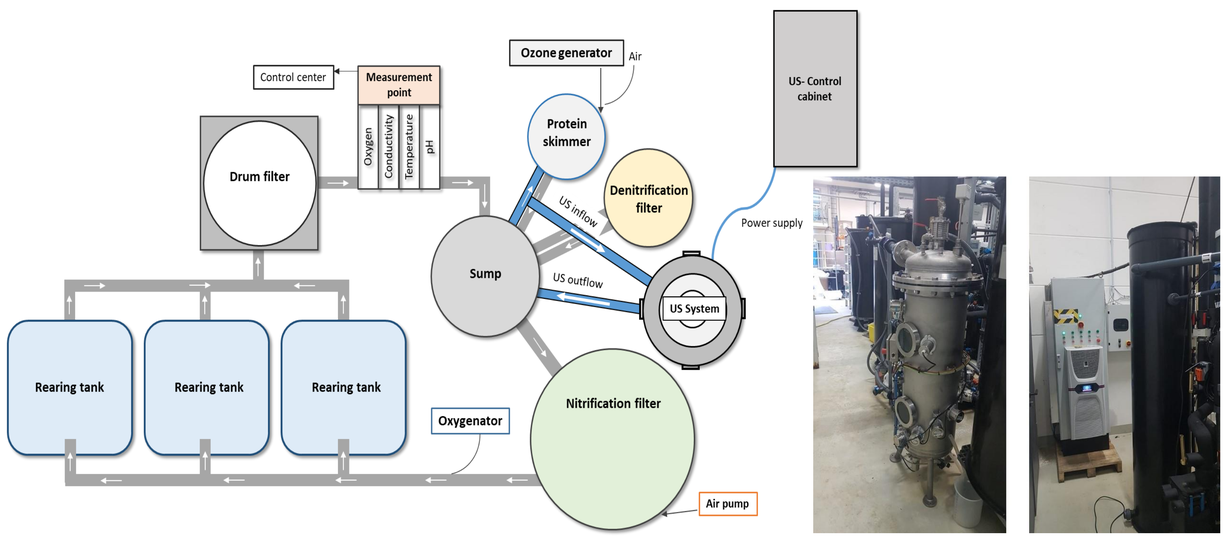
Introduction

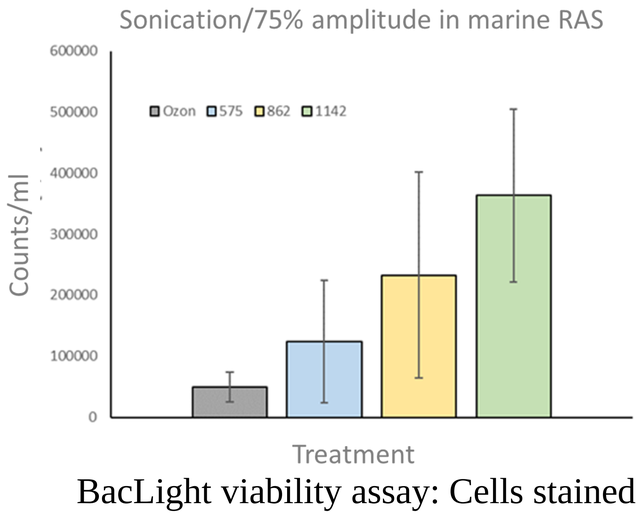
Further development of recirculating aquaculture systems (RAS) towards zero-exchange depends mostly on the improvement of water treatment technologies. Ozone and UV radiation are leading technologies requiring high energy demand and application constrains Sonication is an effective alternative method commonly used in wastewater treatment to eliminate particulate aggregates through cavitation contributing to disrupt bacterial bioflocs and to break microbial cell walls leading to reduced viability. In this study we aim to evaluate the disinfection capacity of a prototype created to treat process water in a RAS in terms of impact on the microbiome of the system as well as bacterial viability was evaluated.

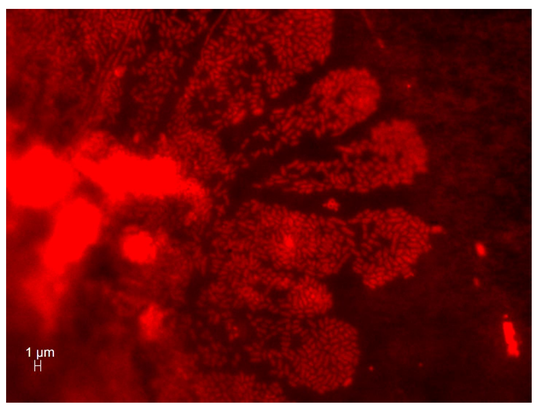
Materials and Methods

- Ultrasound prototype created and adapted to a 5 m3 research RAS composed of three rearing tanks, a drum filter, 2 biofiltration units (nitrification-denitrification), a sump and a protein skimmer with ozone disinfection.
- System initially rearing European Seabass (*Dicentrarchus labrax*) and later on tilapia (*Oreochromis niloticus*).
- Treatment parameters: flow rate:10 l/min; frequencies: 575 kHz, 862 kHz and 1142 kHz without further disinfection. Frequencies amplitude: 50% and 75% by saltwater and 75% by freshwater. Application time by frequency: 96 h
- Daily sampling before first feeding to determine microbial viability (BacLight Viability Kit) and bacterial community composition (FISH) respect to reference untreated samples.
- FISH analysis: FAM labelled DNA probes for Eubacteria (EUB)/Archaea (ARCH) /α-, ß-, γ-, δ-Proteobacteria (ALF, BET, GAM, DELTA) and Actinobacteria (HGC) with respective non-labelled competitors. All samples were counterstained with DAPI.



Ultrasound prototype and control units adapted to a research RAS





BacLight viability assay: Cells stained with PI represent the non-viable population

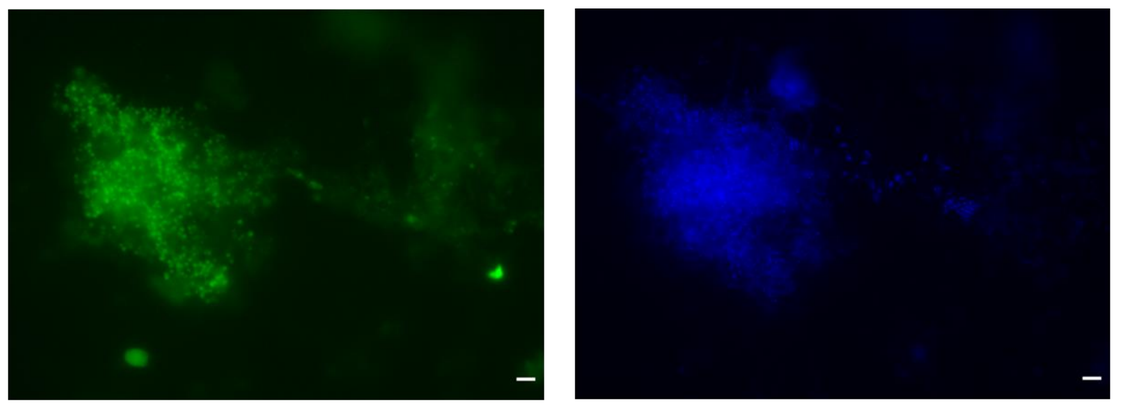
Results and Discussion

Marine RAS:

- Frequency amplitude influences the sterilizing effect: 575 kHz and 1142 kHz showed higher disinfection potential by 75% amplitude than 50%.
- Number of dead/non-viable cells increased with the frequency.
- By 1142 kHz: consequent decrease in the total number of most of the selected bacterial groups was detected
- By 575 kHz and 860 kHz no significant change on total bacterial number.
- Sonication with all tested frequencies lead to changes in the bacterial community. Especially at 1142 kHz, a strong decrease in ALF, BET, GAM and ARC and an increase in DEL was observed (Table 1).
- Selective impact of sonication on microbial community might lead to outgrowing of specific groups.

Freshwater RAS:

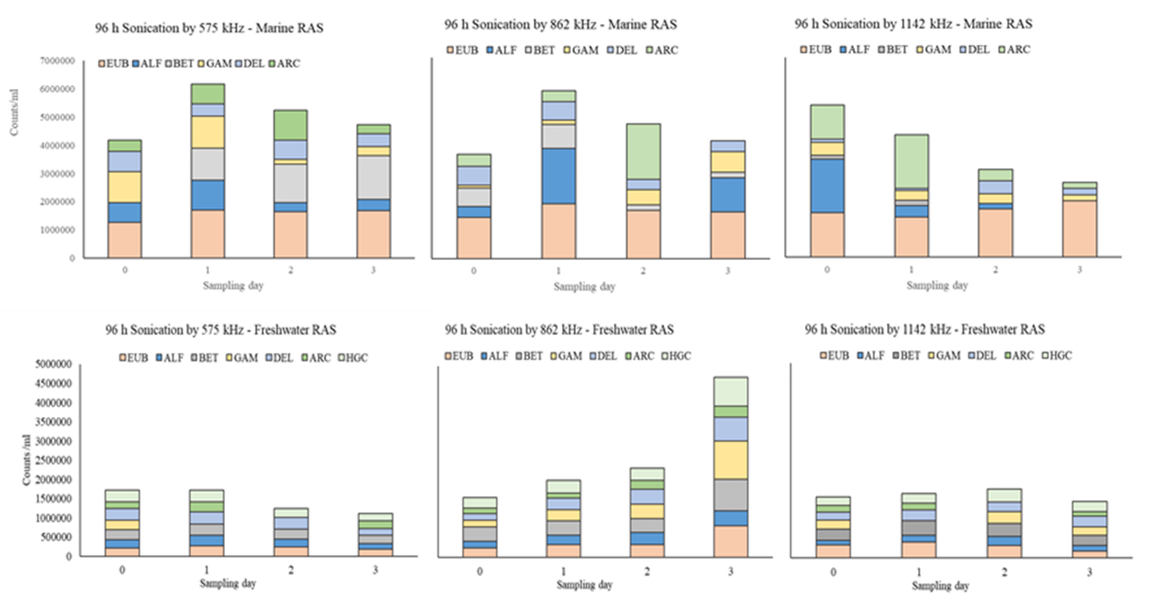
- In freshwater: no defined impact of the treatment on bacterial composition over time
- By 860 kHz: increase in the number of counted bacteria over time
- By 575 and 1142 kHz: slightly drop on bacterial load with no marked changes in community composition
- For all frequencies: no conspicuous change in the number of dead/non-viable cells



FISH assessment: Samples hybridized with DNA probes labelled with FAM fluorophore (in green) and counterstained with DAPI (in blue). Scale bar = 5µm.

Table 1. Temporal change in abundance of selected bacterial groups during US treatment

	Marine RAS				
Frequencies	ALF	BET	GAM	DELTA	ARCH
575 kHz	↓	↑	↓	↓	↓
862 kHz	↑	↓	↑	↓	↑
1142 kHz	↓	↓	↓	↑	↓



Abundance of the different microbial groups determined with FISH

Acknowledgements

This study is part of the BMBF financed project „Ultraschall-Behandlung von Prozesswasser in Aquakultur- sowie Abwasser-Anlagen“ (UvPAA), grant agreement number 02WQ1432B, conducted in cooperation with Meinhardt Ultrasonics, AIMES and GEOMAR. The authors would like to thank Lars Friedlich, Urban Tillmann and Ute Marx for their support with microscopic facilities

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ABSTRACTS

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Introduction

Further development of recirculating aquaculture systems (RAS) towards zero-exchange depends mostly on the improvement of water treatment technologies. Ozone and UV radiation are leading technologies requiring high energy demand and educated staff able to manage the hurdles of their application. Moreover, there are some constrains for systems with poor mechanical filtration or where accumulation of particles higher than 50 μm significantly reduce the penetration potential of UV application. An alternative method commonly used in wastewater treatment to eliminate particulate aggregates is sonication. This method is based on cavitation effects which contribute to disrupt bacterial bioflocs and to break microbial cell walls leading to reduced viability. The present study aims to evaluate the disinfection capacity of a prototype created to treat process water in a RAS rearing aquaculture relevant freshwater and saltwater species with three sonication frequencies. The potential impact on the microbiome of the system in different compartments beside the reactor as well as bacterial viability was evaluated.

Material and Methods

An ultrasound prototype composed of 12 inducers connected to control devices was created in the frame of this project and adapted to a 5 m^3 research RAS composed of three rearing tanks, a drum filter, 2 biofiltration units (nitrification-denitrification), a sump and a protein skimmer with ozone disinfection. For the experiments the system was initially prepared for rearing European Seabass (*Dicentrarchus labrax*) and in a second experiment for rearing tilapia (*Oreochromis niloticus*). Process water coming from the sump was conducted into the prototype at a flow rate of 10 l/min and treated with 575 kHz, 862 kHz and 1142 kHz without further disinfection. For the saltwater experiments we tested 50% and 75% frequencies amplitude while only 75% amplitude was used for freshwater experiments. Each frequency was applied for 96 h and daily sampling was conducted to determine variations on microbial viability (BacLight Viability Kit) and bacterial community composition (FISH) with respect to reference samples collected before treatment. For FISH analysis (Fig. 1) generic FAM labelled DNA probes for Eubacteria (EUB) and Archaea (ARCH) as well as more specific probes for α-, β-, γ-, δ-Proteobacteria (ALF, BET, GAM, DELTA) and Actinobacteria (HGC) were included. When available, also non-labelled competitor DNA probes in equimolar concentration as the respective labelled probe were used. All samples were counterstained with DAPI.

Results and Discussion

Marine RAS:
The sterilizing effect was impacted by the amplitude used. Frequencies 575 kHz and 1142 kHz showed higher disinfection potential by 75% amplitude than 50%. The proportion of dead cells increased with the frequency. At 1142 kHz, a decrease in the total number of most of the selected bacterial groups was detected (Fig. 2) while the total numbers of bacteria at the end of application did not significantly change when using 575 kHz and 860 kHz. Sonication with all tested frequencies lead to changes in the bacterial community. Especially at 1142 kHz, a strong decrease in ALF, BET, GAM and ARC and an increase in DEL was observed (Table 1). This suggests a selective effect of US treatment on microbial community.

Freshwater RAS:
The sonication treatment of the system while rearing freshwater species did not show a defined impact with respect to changes in bacterial composition over time. At a frequency of 860 kHz, there was an increase in the number of counted bacteria over time (Fig. 3) while a slightly drop was observed by 575 and 1142 kHz. No marked changes in the composition of the bacterial community were detected for the latter frequencies. For all frequencies tested there was no conspicuous change in the percentage of dead cells.

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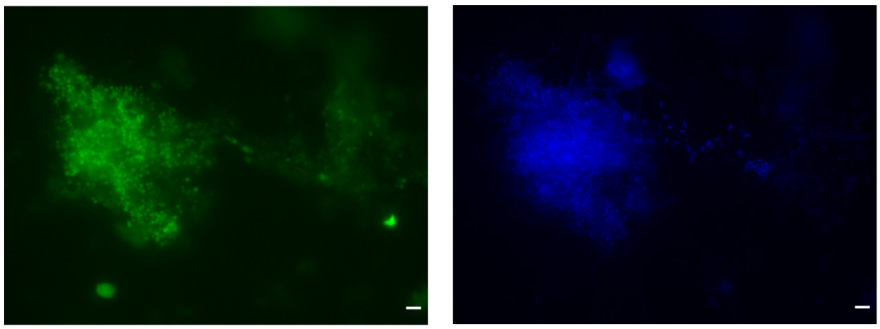

Fig. 1 FISH assessment: Samples hybridized with DNA probes labelled with FAM fluorophore (in green) and counterstained with DAPI (in blue). Scale bar = 5µm.

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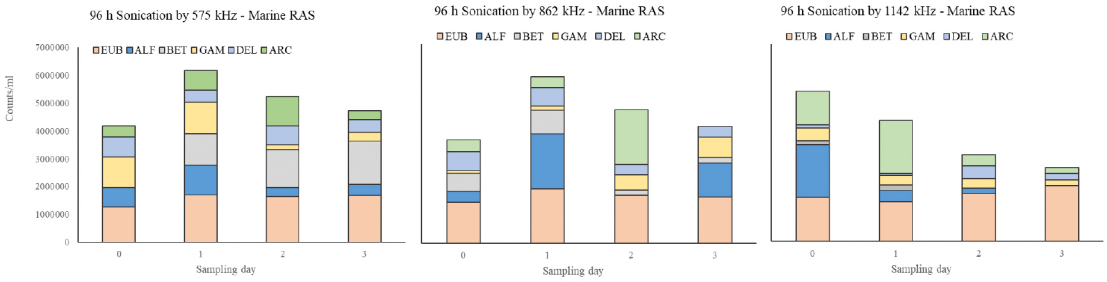

Fig. 2 Bacterial community composition on saltwater samples exposed to three frequencies for 96 h

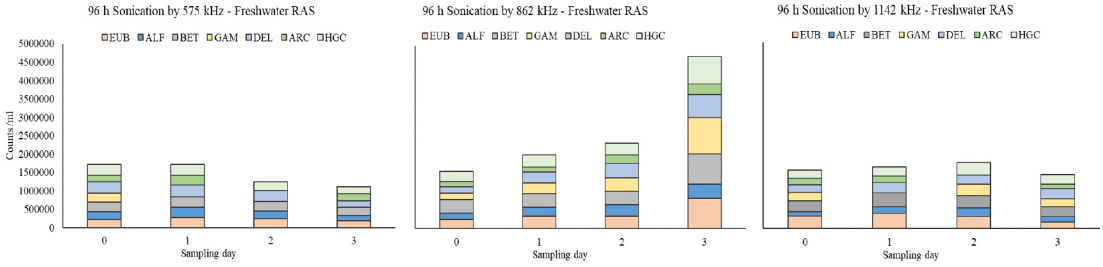

Fig. 3 Bacterial community composition on freshwater samples exposed to three frequencies for 96 h

This study is part of the BMBF financed project „Ultraschall-Behandlung von Prozesswasser in Aquakultur- sowie Abwasser-Anlagen“ (UvPAA), grant agreement number 02WQ1432B