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Dynamics of geosmin-producing bacteria in a full-scale saltwater recirculated aquaculture system

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Abstract

Regular monitoring of the microbiome in recirculated aquaculture systems will allow the farmer to link water quality parameters to subsequent fish performance and will function as a tool for early identification of microbial conditions that could lead to impaired fish health.

Microbial communities were monitored in different compartments of a commercial full-scale, land-based saltwater RAS for production of Atlantic salmon during 9 months. The microbial communities revealed a unique composition in the production tanks and moving bed biofilters, as compared to the other investigated compartments. The fixed bed compartment had a higher stability in the microbial community over time, relative to the moving bed biofilter compartment. Changes of the operation of the facility (replenishment of brackish water to seawater) caused an immediate shift in the microbial populations. Potential geosmin producers (bacteria harbouring the functional gene for geosmin synthetase *geoA*) were dominated by *Sorangium*, *Actinomycetales* and *Myxococcales,* but unidentified microorganisms harbouring the *geoA* gene were also present. The biofilters had the highest numbers of potential geosmin producers, but their presence did not co-vary with the levels of geosmin. This suggest that an observed period with high levels geosmin were due to a change in activity, rather than an increase in cell numbers. Our results provide new insight into the diversity of microbiota in RAS and suggest that monitoring of microbial communities in the aquaculture production may provide a tool for future microbial management to ensure stability in RAS performance.

Keywords

Land-based salmon production

Geosmin-producing microorganisms

Microbial communities

Microbial management

1. Introduction

Geosmin is a secondary metabolite with an earthy off-flavour and is produced by a diverse group of organisms, including the prokaryotic orders *Actinomycetales* and *Myxococcales*, the phylum *Cyanobacteria*, and eukaryotic fungi (Dickschat et al., 2005; Giglio et al., 2008; La Guerche et al., 2005; Lukassen et al., 2017; Yamada et al., 2015). Geosmin has been found to cause off-flavour in a wide range of environments, e.g., drinking water treatment facilities, juice and wine production industries, and in recirculating aquaculture systems (RAS) (Callejón et al., 2016; Hargesheimer and Watson, 1996; Morales-Valle et al., 2010). The human threshold for detection of geosmin is low (4-10 ng/L) and this makes the compound recognizable at even very low concentrations (Nakajima et al., 1996; Watson et al., 2016).

Fish mainly take up geosmin via the gills, but possibly also from the intestinal tract due to ingested, geosmin-producing bacteria, after which the compound accumulate in the adipose tissue (From and Hørlyck, 1984; Howgate, 2004). While geosmin rapidly absorbs and accumulates in the fish, depuration of the compound from fish is a slow process that requires several days to reach a level below human perception, typically below 250 ng/kg fish (Burr et al., 2012; Howgate, 2004; Petersen et al., 2011). Depuration of fish in aquaculture farms is a laborious process that requires a large volume of off-flavour-free water and may also cause weight loss due to absence of feeding during the depuration. Thus, depuration of geosmin may inflict an economic loss to the industry (Hanson, 2003; Tucker, 2000).

Microbiological research in RAS has typically focussed on prevalence of pathogens and importance of ammonia-oxidising microorganisms, while knowledge on dynamics of the microbial communities in RAS is scarce (Bentzon-Tilia et al., 2016). Better knowledge on microbial processes and the involved microorganisms in RAS may help optimizing environmental parameters to ensure a

healthy fish production (Bergheim et al., 2009), and maintain low occurrence of geosmin and other microbial off-flavours. Specific groups of geosmin-producing microorganisms and environmental factors impacting their abundance have been identified in RAS, e.g., in rainbow-trout production (Auffret et al., 2013; Sarker et al., 2014; Schrader et al., 2013). However, these studies almost exclusively present pilot-scale surveys or single sampling point observations in full-scale systems that may not reflect long-time variations in composition of microorganisms in an operative, commercial RAS.

Here, we describe microbial dynamics in a full-scale saltwater RAS during production of Atlantic salmon over a 9 months period. Composition of the microbial communities was characterized by 16S rRNA gene sequencing and related to the presence of putative geosmin-producing microorganisms and occurrence of geosmin in the water. The aim of the study was to provide knowledge on the microbial community composition in different compartments of the full-scale RAS facility to better understand how microbial dynamics are involved in the production of geosmin.

2. Materials and methods

2.1. Study site and sampling

The present study was carried out during a period of 41 weeks in an Atlantic salmon (*Salmo salar*) indoor RAS facility located in western Jutland, Denmark. Saltwater used in the RAS was initially taken from the nearby brackish water Ringkøbing Fjord, but in week 25 salt water was pumped into the facility from the North Sea and approximately 20 % of the total RAS volume was replenished with this water source. Salinity of water in the RAS was kept at 21 ppt. The facility consists of fourteen production tanks, with volumes ranging from 250 m^3 to 870 m^3 and with a total system flow of 7200 m^3/h. From the production tanks, water is directed into drum filters, followed by two separate biofilters; the first biofilter has a fixed bed while the second filter has a moving bed (Fig. 1). After the moving bed biofilter, the water enters a trickling filter for degassing, before 4.17% of the total system flow enters a final UV treatment and returns into the production tanks. After week 13, an ozone unit and a foam-fraction system was installed and the UV treatment step was removed. Except for the

ozone unit and the change of water inlet, operation of the RAS remained unchanged with respect to flow rate, particle filtration, ventilation and light regime throughout the sampling period.

Water samples were collected from two production tanks (T1 and T2) at intervals of 1-3 weeks during the sampling period. As fish were growing, they were transferred twice to larger tanks (week 4 and week 17), and therefore the subsequent sampling was done in the new (and clean) tank to which the fish were moved. In addition to production tanks, water samples were also collected before and after the drum filter, after the UV treatment unit, and within the moving and fixed bio-filtration beds. Water samples (approximately 80 mL) were filtered through 47 mm diameter 0.45 µm pore size cellulose ester membrane filters (Merck Millipore). Biofilter samples were collected as individual plastic carriers and biofilm samples at randomly selected places in the two biofilters. Material on the carriers was removed by cutting the carriers into small pieces using a sterile scalpel and conduct bead-beating directly on these pieces. Fixed bed samples were sampled by carefully scraping off the biofilm using a cell scraper (Sarstedt), and simultaneous collecting water from rinsing the membrane was collected using 50 mL syringe and filtered together with the biofilm onto a filter as described above. All samples were kept at -20 °C until further analysis. For determination of geosmin concentrations, 50 mL water was sampled from the production tanks and after the drum filter in completely filled glass bottles (no head space). The bottles were closed using airtight caps with a teflon inlay and kept at -20 °C until further analysis.

2.2. Geosmin analysis

Concentrations of geosmin were determined by stir bar sorptive extraction (SBSE) and GC-MS detection as described elsewhere (Petersen et al., 2014). Briefly, a commercial stir bar at 1.00 cm length and thickness of 1.00 mm (Twister TM) coated with polydimethylsiloxane (PDMS), was added to 10 mL of water in a 10 mL glass vial. SBSE was carried out at room temperature by shaking at 1,000 rpm for 120 min. After extraction, the twisters were removed with forceps, rinsed with water, dried with lint-free tissue and transferred to thermal desorption tubes. A calibration curve was

prepared from 1, 10, 50, 100, and 1000 ng/L geosmin in water and used for quantification (R^2 = 0.99) of geosmin in the water.

2.3. DNA extraction and quantification of geoA

The filters with particles from the water and biomass from the fixed bed were divided into three equal pieces and DNA was extracted in triplicates using the Fast-Prep soil kit (MP Biomedicals). After bead-beating for 2 × 40 s at 6 m/s in FastPrep model FP120 (MP Biomedicals), instructions by the manufacturer of the Fast-Prep soil kit were followed. Plastic carriers from the moving bed biofilter were carefully cut into small pieces under sterile conditions and transferred to bead-beating tubes in triplicates. DNA was extracted from the carrier fragments using the Dneasy Powersoil DNA isolation kit (Qiagen) following the recommendations of the manufacturer.

Quantification of the *geoA* gene by quantitative PCR (qPCR) was conducted on selected samples using either specific primers targeting *geoA* in *Actinomycetales*, *Myxococcales*, *Sorangium*, or universal *geoA* (Cyc) primers and qPCR conditions as described in details elsewhere (Ludwig et al., 2007; Lukassen et al., 2017).

2.4. Amplicon sequencing, bioinformatics and statistical analyses

Microbial communities in the water and the biofilter samples were characterized by amplicon sequencing of the V1-3 region of the 16S rRNA gene using an Illumina MiSeq platform. All sequenced sample libraries were subsampled to 50,000 raw reads and screened for PhiX contamination using bowtie2 v. 2.1.0 with standard settings and all matching reads removed (Langmead and Salzberg, 2012). Sequences were trimmed with Trimmomatic 0.32 to ensure that the average quality per base is above 3 (Bolger et al., 2014). The reads were dereplicated, chimeric reads removed and formatted for use in the UPARSE workflow (Edgar, 2013). The reads were clustered, and OTU abundance at species level (97% identity) was estimated using usearch v. 7.0.1090 with default settings. Taxonomy

was assigned with the RDP classifier (Wang et al., 2007), using MiDAS taxonomy version 1.20 and the SILVA taxonomy (McIlroy et al., 2015; Quast et al., 2013).

All statistical analyses were performed in RStudio (www.rstudio.com) using R version 3.3.2 (R Core Team, 2016). Principal component analysis (PCA), heatmaps and level plots were conducted using the R packages ampvis (v1.26) (Albertsen et al., 2015), ggplot2 (Wickham, 2009) and Phyloseq (McMurdie and Holmes, 2013). Constrained redundancy analysis (RDA) was applied to the sequence data to identify correlations between geosmin and OTU abundances. Microbial richness and evenness were determined using Chao1 and Shannon-Weaver indices and compared by the Kruskal-Wallis test in the R package.

3. Results

3.1. Geosmin in tank water

Geosmin concentrations were measured in the production tanks T1 and T2 and after the drum filter (A) (see schematic presentation of the RAS facility in Fig. 1). In both tanks, the concentrations were highest during the initial weeks, ranging from 112.7 to 169.2 ng/L in T1 in weeks 3 to 11, and from 105.7 to 235.5 ng/L in T2 in week 3 to 15 (Fig. 2). In the remaining weeks of the sampling period, the concentrations were below 100 ng/L in both tanks. Samples taken immediately after the drum filter had a significantly lower content of geosmin (One-way ANOVA, $p < 0.001$) of below 5 ng/L during week 3 to 10 and between 10.1 to 48.9 ng/L in week 11 to 41.

3.2. geoA copy numbers

Quantification of copy numbers of the *geoA* gene in *Actinomycetales*, *Myxococcales* and *Sorangium* and the universal *geoA* gene in water of T1 and T2 showed that (1) abundance of the universal *geoA* gene ranged from 15,455 to 296,247 copies/mL; (2) copy numbers of *geoA*-affiliated *Actinomycetales*, *Myxococcales* and *Sorangium* ranged from none to >8,800 (for *Sorangium*) with

mean values of 558, 110 and 611 *geoA* copies/mL, respectively; (3) relative to the total population, as estimated by the universal 16S rRNA gene copy numbers, *geoA* in *Myxococcales* made up an average of 0.1 %, while *geoA* in *Actinomycetales* and *Sorangium* each made up an average of 0.6 % (Table 1). In the moving bed biofilter carriers, *geoA*-affiliated *Actinomycetales* and *Sorangium* were detected on all carriers with mean *geoA* copy numbers of 19,140 and 80,036 per carrier. Relative to copies detected with the universal primers (average of 300,220 *geoA* copies/carrier), *geoA*-affiliated *Actinomycetales* and *Sorangium* made up 6.3 and 17 %, respectively. Thus, more *geoA* copies could be associated with the three taxonomic groups on the biofilter carriers (average of 41 %, n=10) than in the water (average 1.2 %, n=18). Although the nature of the qPCR data indicate large dynamic and/or technical variations, it does not cover any statistical significant changes over time in neither the water nor on the carriers, as estimated by Spearman rank correlation analysis.

3.3. Microbial communities in water and biofilters

The microbial communities were characterized by 16S rRNA gene sequencing of 151 samples. A total of 4,207 taxa were successfully identified across all samples and 5,480,788 sequences were obtained. At least 5,000 sequences from each sample were used for further analysis. Alpha diversity (number of operational taxonomic units, OTU) at the different sites within the aquaculture facility (see Fig. 1 for sampling sites) were 969 ± 209, 1,121 ± 142, 1,301 ± 144 and 1,200 ± 174 for (1) production tank T1 and T2, (2) water from the fixed bed (site B), (3) the moving beds (site C) and (4) water from the sampling sites A, D and E, respectively.

The microbial composition in water of T1 and T2 was dominated by three phyla, which together made up approximately 95 % of the total read abundance: *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* (Fig. 3). *Proteobacteria* constituted 25-91 % of the total reads with the orders *Rhodobacterales* and *Burkholderiales* as the most dominant OTUs. In the moving bed biofilters, the dominant phyla were *Proteobacteria* and *Bacteroidetes*, making up about 90 % of the total read abundance, and with *Rhodobacterales*, *Flavobacteriales* and *Sphingobacteriales* as the dominant orders. In the water from the fixed bed biofilter, the dominant phyla were similar to those for the

moving beds, but had a larger contribution of the *Actinobacteria*, and together the three phyla made up 90% of the total read abundance. In the fixed bed, the major orders were, like in the moving bed, *Rhodobacterales, Flavobacteriales* and *Sphingomonadales.* The *Myxococcales* order constituted 1.6-2.4 % of the total read abundance in the moving bed biofilters, but only 0.3-0.8 % in the fixed bed. For *Actinobacteria*, the largest difference in abundance occurred between samples collected from the biofilters (B and C) and the fish production tanks (T1 and T2).

3.4. Diversity of microbial communities

Multivariate statistics analysis of all the collected sequence information (ordination plot), revealed that tank T1 and T2 clustered together, while microbial communities in the moving bed biofilter (C) formed a distinct and dense cluster, separated from T1 and T2 along the secondary principal coordinate axis (Fig. 4). The widespread distribution of the microbial communities in T1 and T2, relative to the biofilter samples, indicated a larger variation over time in the tank communities (Fig. 4), although communities in T1 and T2 were still more similar to each other than rest of the sample types (permutation test, $p < 0.001$). Water from the fixed bed biofilters (B), the trickling filter (D), after the drum filter (A) and UV treatment (E) all clustered together. The clustering of the different sample types was statistically significant as estimated by a permutation test ($p < 0.001$).

The sequencing data had a high reproducibility, as indicated by the repeated sampling of selected data, and this makes it possibly to examine temporal population dynamics (Fig. 5). In T1 and T2, analysis of variations in the microbial communities during the sampling period revealed that the communities exhibited similar changes over time. Redundancy analysis of the microbial communities, using the level of geosmin measured in the water samples as a constrained factor, revealed a time-dependent change that showed a transfer from relative high geosmin levels towards low levels (Fig. 5). The strongest correlation (loading) among the bacterial taxa did not encompass known geosmin producers, and only members of the geosmin-producing order *Frankiales* were among the top 50 highest loading. The population dynamics showed a clear time-dependent

trajectory along the secondary ordination for the moving bed biofilter samples (C), while the microbial dynamics showed minor drift in the samples collected in the fixed bed (Fig. 6). Two large shifts in the population dynamics, along this ordination axis PC2, occurred when the combined ozone treatment and foam fractionation system was installed in week 13, and after replenishment of 20 % of the water by fresh seawater after week 25 (Fig. 6).

4. Discussion

4.1. Geosmin and geosmin producing microorganisms

Identification of bacterial communities and quantification of *geoA* genes in different compartments of the present RAS provides insight into the distribution of potential microbial geosmin producers and may help predict the presence of hotspots and potential factors important for enhanced geosmin concentrations in different compartments of the salmon production facility. During the nine months sampling period, it was observed that the overall level of geosmin was highest in the two production tanks. Immediately after filtration in the micro-sieve part of the drum filter, most of the geosmin was removed, and 5 to 6-fold lower concentrations of geosmin were found in the water sampled after the drum filter, relative to the water in the production tanks (Fig. 1). Since the residence time of water in the drum filter is very short (few seconds), the reduction in geosmin was most likely caused by retention of particles to which geosmin was absorbed or by evaporation from the water. Sorption of geosmin to sludge particles in a RAS facility has previously been reported (Guttman and van Rijn, 2009).

Concentrations of geosmin in the RAS facility were not reflected in the number of geosmin producers, as estimated by the number of *geoA* gene copies. On the contrary, the *geoA* numbers slowly increased throughout the sampling period, while the geosmin concentrations declined. This suggests an increasing presence of inactive geosmin producers. Surprisingly, most of the potential geosmin-producing microorganisms could not be targeted by the taxon-specific *geoA* primers for *Actinomycetales*, *Myxococcales* and *Sorangium*, suggesting presence of new and unidentified

geosmin producers. The abundance of *geoA* copies in the biofilter evolved quite differently to the water from the production tanks and followed, to some extent, the geosmin levels. In contrast to the production tanks, the *geoA* sequences in the biofilter were shown to affiliate with *Actinomycetales* and *Sorangia*. The high abundance of identified geosmin producers in the biofilter, relative to the water phase, may indicate that the geosmin found in the production tanks derived from the biofilter, rather than being produced in the water. If so, this contradicts other studies conducted with the same primer sets (Lukassen et al., 2017).

4.2. Microbial diversity

The alpha diversity of the identified microbial communities showed that the production tanks had a lower diversity (969 ± 209) than the moving bed biofilter (1301 ± 144). In other aquaculture systems for rearing of salmon and sole, the biofilters were also found to harbour a higher diversity than water in the rearing tanks (Martins et al., 2013; Rud et al., 2016). For the salmon farm, the diversity was higher than found in the present study (approximately 1,200-3,500 in the water and approximately 3,000-6,000 in the biofilters; Rud et al., 2016), while the sole rearing system had a diversity between 450 and 550 (Martins et al. 2013). In earthen carp ponds in China, species richness in the range of 867-1,306 were reported (Qin et al., 2016), and this is comparable to the present variation in diversity in the salmon breeding facility. Possibly, these observations in phylogenetic diversity of the aquaculture microbiota indicate a system-dependence which is determined by abiotic, rather than biotic factors, but also by dispersal effects as also reported previously (Langenheder et al., 2016). In support of abiotic factors being important in shaping the microbial communities, an immediate change in the microbial populations occurred in the present salmon farm, when 20 % of the low salinity fjord water was replaced by fresh seawater (Fig. 6). Obviously, the fresh seawater had a significant impact on the microbial diversity and changed the populations that had established during the initial 24 weeks of intense salmon rearing. A similar effect of water exchange has been observed in a salmon farm (Rud et al., 2016).

The microbial communities grouped into three clusters, comprising one cluster with the moving bed biofilters (C), one cluster with the two production tanks (T1 and T2), and one cluster with water collected after the drum filter (A), after the UV unit (E) and trickling filter (D) (Fig. 4). The diversity in the microbial communities from the different sampling types was statistically significant from each other (permutation test, $p < 0.001$). The microbial composition in the two tanks was similar to each other at each sampling point, but they fluctuated during the 9 months sampling period. The parallel succession of the tanks suggested that the community structure to a large degree depended on the overall operational management, such as feeding rate, water flow, salinity, fish size, etc. (Fig. 5). The microbial composition in the moving bed biofilters also fluctuated after week 13, when the ozone foam fraction system was installed and after week 25, when a fraction of the water was changed (Fig. 6). Ozonation has previously been shown to affect the microbial community in fish tanks (Wietz et al., 2009) and this was supported by the significant community change that occurred in the present salmon farm after implementing of ozonation. In contrast to the microbial populations in water in the tanks and the moving bed biofilter, water from the fixed bed biofilter compartment had a stable microbial community. It was expected that the two biofilter types both had a high stability, but only the fixed bed compartment showed such a consistency.

4.3. Bacterial taxa in the RAS facility

The most abundant phyla in the production tanks (T1 and T2) and the fixed bed biofilter compartment (C) were *Proteobacteria*, *Bacteroidetes* and *Actinobacteria*, while the *Proteobacteria* and *Bacteriodetes* phyla were dominant in the moving bed biofilters (Fig. 3). The orders with the highest read abundance were *Rhodobacterales*, *Burkholderiales*, *Flavobacteriales*, *Sphingomonadales* and *Sphingobacteriales*. These phyla and orders have previously been found in RAS and other aquatic environments (Llirós et al., 2014; Martins et al., 2013; Rud et al., 2016; Sugita et al., 2005). The order *Myxococcales*, which comprise known geosmin-producing species, was found in the moving bed biofilters and in some samples from the fixed bed compartments. Species within this order could be a

potential source of geosmin problems in this RAS (Auffret et al., 2013; Dickschat et al., 2005). No *geoA* nor 16S rRNA genes affiliating to the cyanobacteria were detected in the RAS, which is likely due to the low light intensity in the indoor RAS.

The *redundancy* analysis (RDA), constrained for the geosmin in both fish production tanks, showed a clear trajectory from relatively high loading initially and peaking at week 7-8 when the level decreased (Fig. 5). The taxonomic groups with a strong correlation (loading) for the geosmin-observed trajectory have not previously been associated to geosmin production and thus, they more likely represent abundant organisms with similar fluctuation patterns in the systems. The data showed a clear shift from the highest geosmin levels in the start of the sampling period, to low geosmin levels towards the end. This indicates that certain conditions in the RAS may favour for geosmin production, as well as shaping the entire microbial community.

The present study provided insight into microbial communities that are present in a modern RAS production of salmon in seawater. The microbial communities in the present RAS showed that different compartments within the facility harboured specific bacterial populations, including also specific geosmin producers, e.g., in water in the production tanks, in water after the UV treatment, and in the biofilters. This suggest that environmental drivers, such as nutrients, oxygen content, salinity, etc. were relatively invariable and allowed development of specific microbial communities. The results also showed that microbial populations in the facility were dynamic and changed over time, and they indicate that the community composition appears to reflect the surrounding environment. Thus, this is a first criterion for future application of microbial management as a tool for improved management and optimisation of operations in RAS.

While the major parameters controlling RAS performance from the operational point of view are relatively well understood, insufficient knowledge is available on how the microbial communities impact the health and yield. The microbial communities are of great importance for decreasing outbreaks of pathogens, increased removal of waste products (e.g. geosmin), uptake of nutrients, impact on fish health etc.

Although our observations were only based upon a single study in a full-scale RAS, they suggest that monitoring of microbial communities in aquaculture farms over extended periods may provide a new opportunity for a more direct approach in maintaining and optimizing stability in RAS performance, e.g. with respect to fundamental biological, and physical and chemical processes. Such microbial management should involve identification of novel indicator species that reflect RAS performance and fish health and provide a direct tool for early acting to emerging problems, performance and yield. The data in this study might provide the basis for creating monitoring tools for the RAS operator in the future.

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Table 1: Copy number of the geoA gene in Actinomycetales, Myxococcales, Sorangium and of the universal geoA gene in tank 1 and 2 and the moving bed biofilter. Copy numbers in the water phase in the tanks are shown as copies/mL while copy numbers for the biofilter are shown as copies/plastic carrier. Sampling date indicates weeks after experimental start up. Nd: Not determined. The quantification data are shown as average values based on triplicates.

Sampling site	Date (week)	*Actinomycetales*	*Myxococcales*	*Sorangium*	Universal *geoA* copies	Ratio of *geoA* *
Tank 1	W1	13	73	173	38969	0.19
Tank1	W2	16	20	0	15455	0.06
Tank 1	W3	134	34	0	55400	0.11
Tank 1	W4	61	14	0	40671	0.05
Tank 1	W7	216	53	0	56507	0.03
Tank 1	W8	0	228	0	49681	0.05
Tank 1	W9	966	352	86	65957	0.06
Tank 1	W11	6155	0	84	169113	0.08
Tank 1	W13	72	0	0	296247	0.17
Tank 2	W1	20	0	20	20676	0.05
Tank 2	W2	2295	27	128	78548	0.04
Tank 2	W3	59	1007	0	97136	0.03
Tank 2	W4	13	7	56	61457	0.07
Tank 2	W7	8	0	1148	68757	0.02
Tank 2	W8	15	3	487	29984	0.05
Tank 2	W9	4	12	8824	167609	0.10
Tank 2	W11	0	135	0	174517	0.07
Tank 2	W13	0	10	0	225187	0.06
Biofilter	W1	1304	Nd	71566	113102	0.19
Biofilter	W2	28	Nd	75225	102007	0.18
Biofilter	W3	1592	Nd	145575	104538	0.09
Biofilter	W4	3701	Nd	7880	98287	0.59
Biofilter	W7	112	Nd	1396	72878	0.66
Biofilter	W8	46619	Nd	10549	94179	0.22
Biofilter	W9	413	Nd	19748	305074	0.75
Biofilter	W10	523	Nd	7369	196443	0.31
Biofilter	W11	3562	Nd	20726	284460	0.18

Biofilter	W13	37841	Nd	40141	130134	0.09

* Ratio of *geoA* copies per 16S rRNA gene copies indicate a normalized ratio of the two gene copy numbers from the same biomass

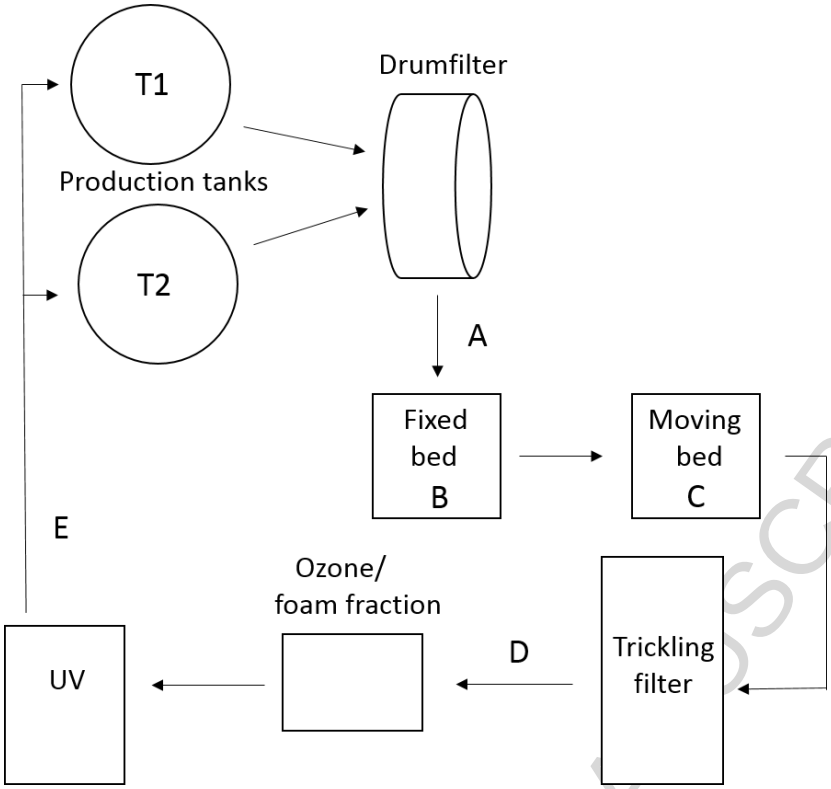

Fig. 1: *Schematic overview of the RAS including sampling points. A: After drum filter. B: Water from the fixed. C: Moving bed biofilter compartment. D: After trickling filter. E: After UV unit. T1: Fish production tank 1. T2: Fish production tank 2.*

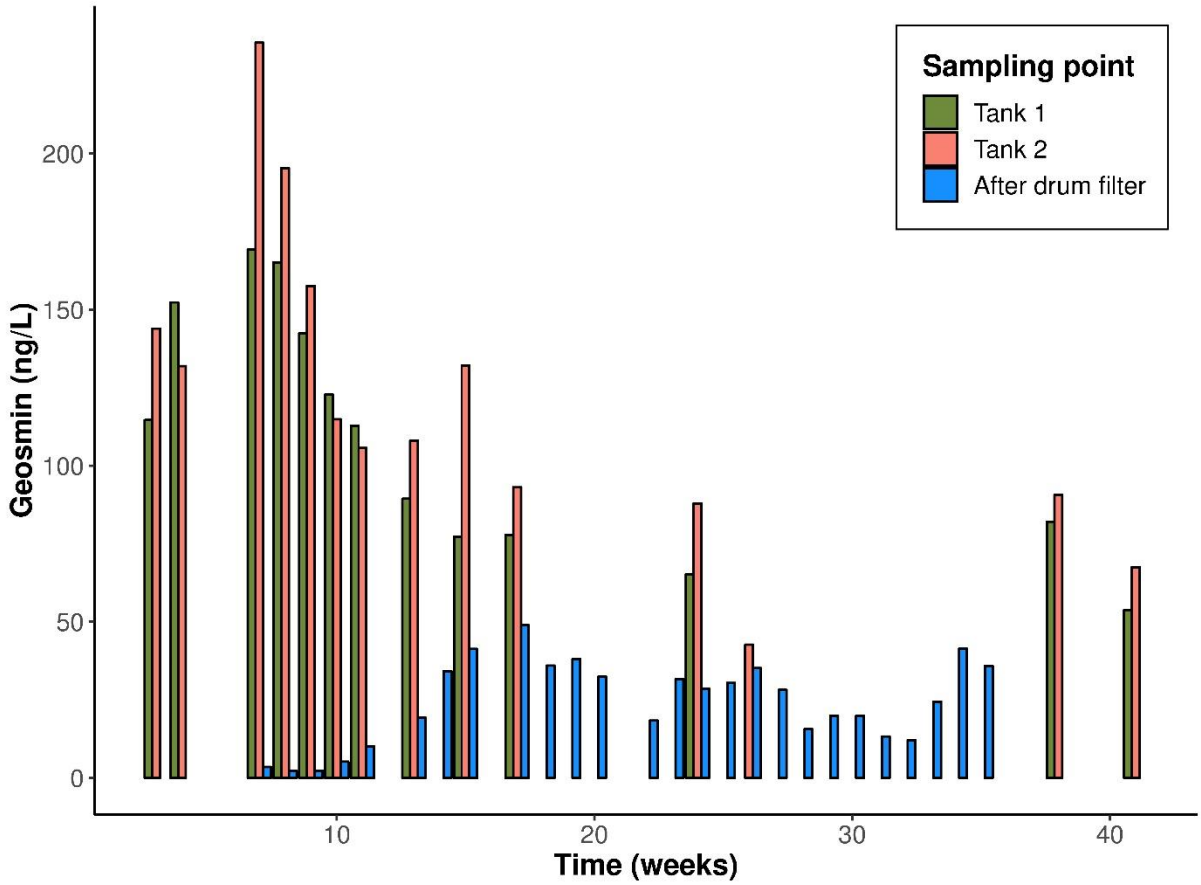

Fig. 2: *Geosmin concentrations [ng/L] in the two fish production tanks and water sampled after the drum filter. Nd: Not determined.*

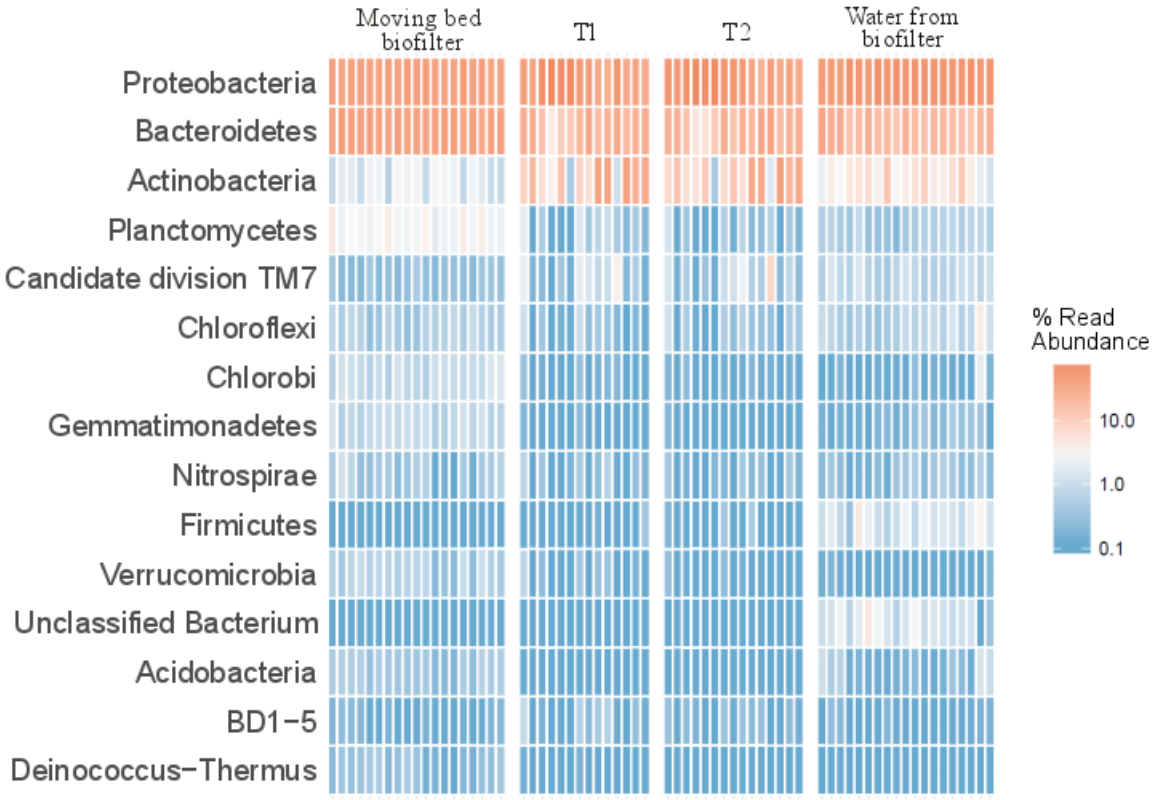

Fig. 3: *Heatmap of the 21 most abundant phyla in the moving bed (C), tank 1 (T1), tank 2 (T2) and water from the fixed bed compartment (B). Sampling times were as follows. Moving bed biofilter: Week 1, 2, 3, 4, 7, 8, 9, 10, 11, 13, 15, 17, 21, 23, 25, 37, 38, 39 and 41; T1: Week 1, 2, 3, 4, 7, 8, 9, 10, 11, 13, 15, 17, 24, and 41; T2: Week 1, 2, 3, 4, 7, 8, 9, 10, 11, 13, 15, 17, 24, 26 and 41; Water from fixed biofilter: Week 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 22, 23, 24, 25, 30, 31, 32 and 33.*

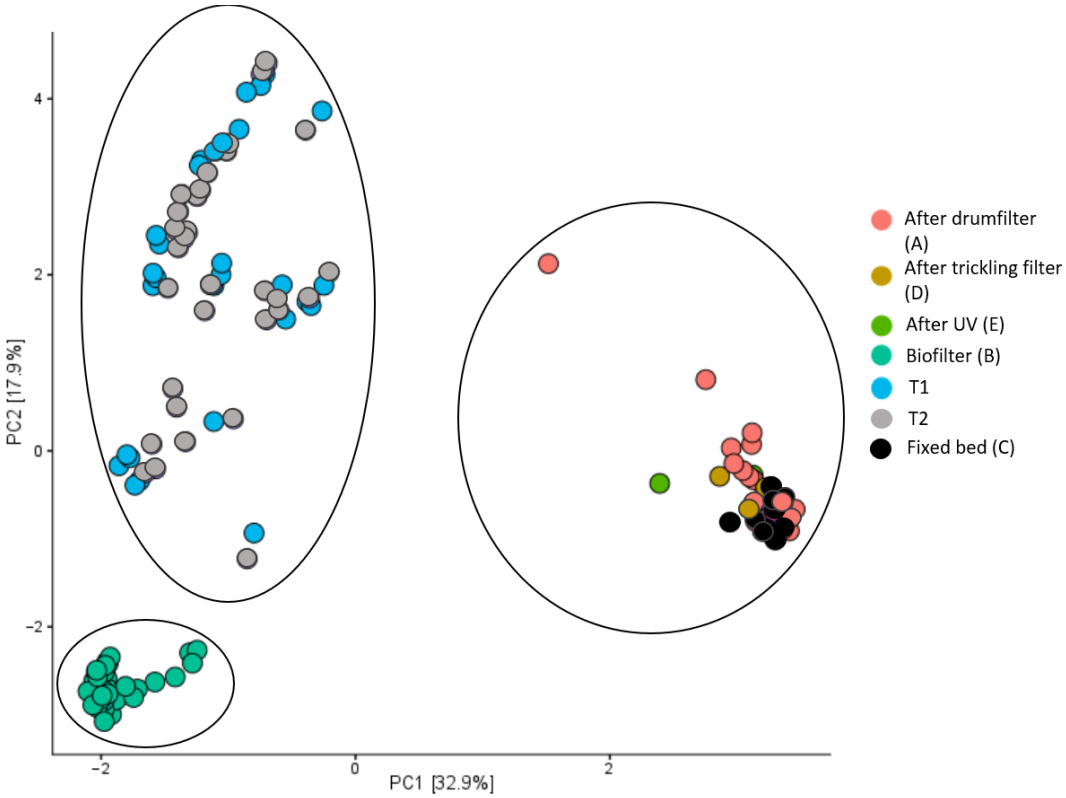

Fig. 4: *PCA plot of distances between microbial communities sampled in the different RAS compartments. Each point represents all sequences from a sample collected in the indicated compartment.*

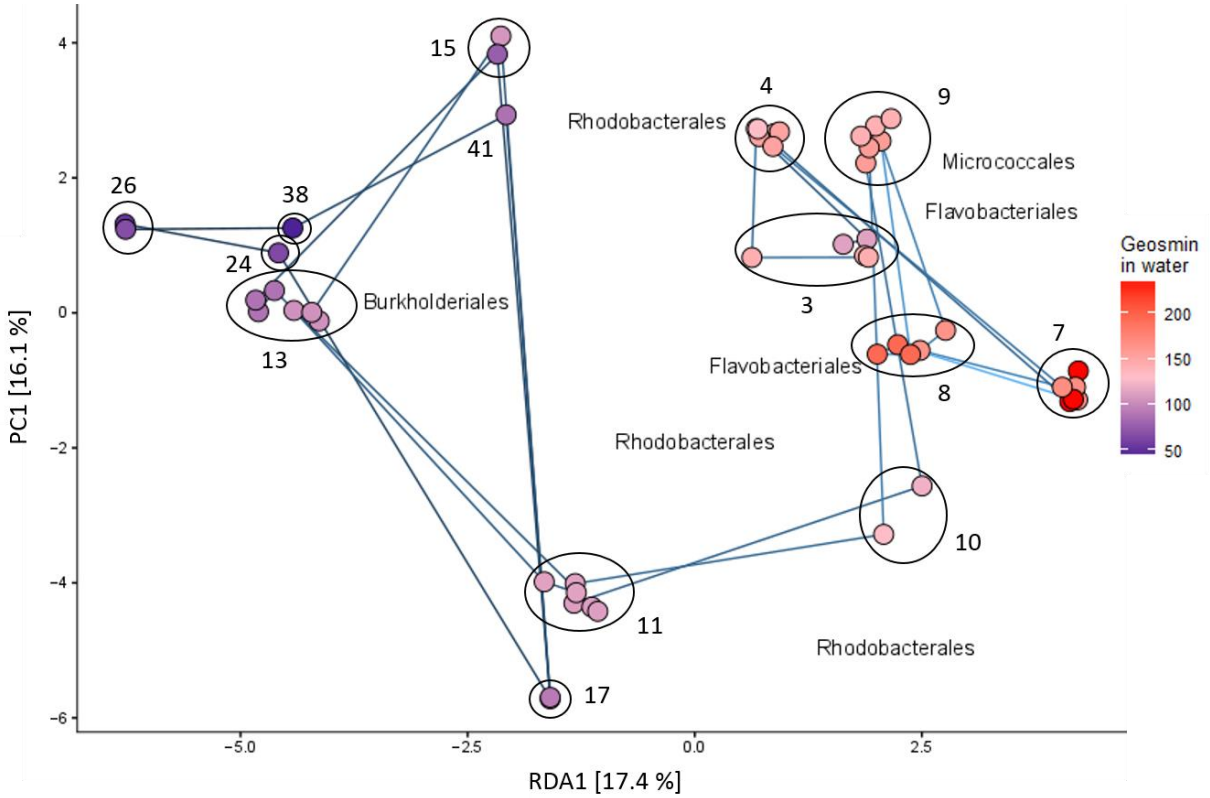

Fig. 5: *Constrained RDA plot showing the linear relationship between the geosmin content in the water in production tank T1 and T2 (in ng/L) and the OTU abundance during the 41 week sampling period. Numbers in the plot refers to the sampling weeks. The five OTUs with the strongest correlation (loading) are shown in the plot with the highest possible taxonomic resolution.*

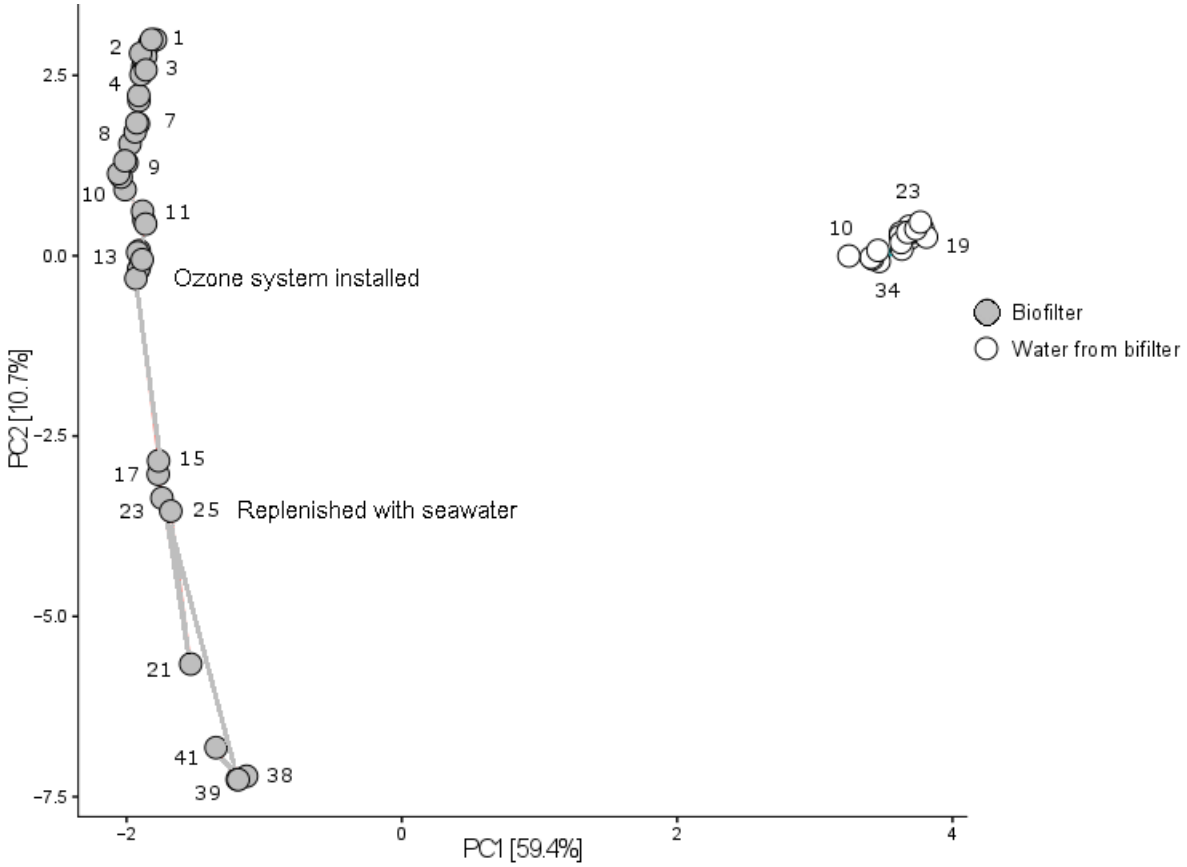

Fig. 6: *PCA plot showing the development of the microbial community in the moving bed biofilters (C) and in water from the fixed bed biofilter compartment (B). The numbers refer to sampling weeks.*

Highlights:

- Monitoring of microbial communities were observed in a full-scale saltwater RAS over a period of 9 months
- *Sorangium, Actinomycetales* and *Myxococcales* were identified in the system as important geosmin producers but also unidentified geosmin-producing bacteria were present
- The presence of potential geosmin producers did not coincide with the presence of geosmin levels, indicating the increased levels were due to changes in activity rather than abundance
- Microbial communities reflects changes in operation indicates the importance of microbial management as a tool for improved aquaculture performance