JYX JYVÄSKYLÄN YLIOPISTO UNIVERSITY OF JYVÄSKYLÄ

This is a self-archived version of an original article. This version may differ from the original in pagination and typographic details.

Author(s): Lindholm-Lehto, P. C.; Suurnäkki, Suvi; Pulkkinen, Jani; Aalto, Sanni L.; Tiirola, Marja; Vielma, J.

Title: Effect of peracetic acid on levels of geosmin, 2-methylisoborneol, and their potential producers in a recirculating aquaculture system for rearing rainbow trout (Oncorhynchus mykiss)

Year: 2019

Version: Accepted version (Final draft)

Copyright: © 2019 Elsevier Inc

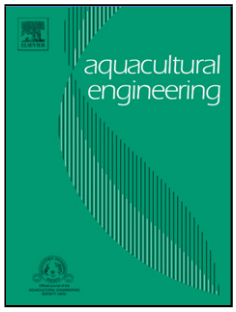
Rights: CC BY-NC-ND 4.0

Rights url: https://creativecommons.org/licenses/by-nc-nd/4.0/

Please cite the original version:

Lindholm-Lehto, P. C., Suurnäkki, S., Pulkkinen, J., Aalto, S. L., Tiirola, M., & Vielma, J. (2019). Effect of peracetic acid on levels of geosmin, 2-methylisoborneol, and their potential producers in a recirculating aquaculture system for rearing rainbow trout (Oncorhynchus mykiss). Aquacultural Engineering, 85, 56-64. https://doi.org/10.1016/j.aquaeng.2019.02.002

Accepted Manuscript



Title: Effect of peracetic acid on levels of geosmin, 2-methylisoborneol, and their potential producers in a recirculating aquaculture system for rearing rainbow trout (*Oncorhynchus mykiss*)

Authors: P.C. Lindholm-Lehto, S. Suurnäkki, J.T. Pulkkinen, S.L. Aalto, M. Tiirola, J. Vielma

PII: S0144-8609(18)30144-4
DOI: https://doi.org/10.1016/j.aquaeng.2019.02.002
Reference: AQUE 1986

To appear in: *Aquacultural Engineering*

Received date: 18 October 2018
Revised date: 14 February 2019
Accepted date: 28 February 2019

Please cite this article as: Lindholm-Lehto PC, Suurnäkki S, Pulkkinen JT, Aalto SL, Tiirola M, Vielma J, Effect of peracetic acid on levels of geosmin, 2-methylisoborneol, and their potential producers in a recirculating aquaculture system for rearing rainbow trout (*Oncorhynchus mykiss*), *Aquacultural Engineering* (2019), https://doi.org/10.1016/j.aquaeng.2019.02.002

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Effect of peracetic acid on levels of geosmin, 2-methylisoborneol, and their potential producers in a recirculating aquaculture system for rearing rainbow trout (*Oncorhynchus mykiss*)

P. C. Lindholm-Lehto[a*], S. Suurnäkki[b], J. T. Pulkkinen[a,b], S. L. Aalto[b,c], M. Tiirola[b], J. Vielma[a]

Contact information: [a]Aquatic Production Systems, Natural Resources Institute Finland (Luke), Survontie 9A, FI-40500 Jyväskylä, Finland

[b] University of Jyväskylä, Department of Biological and Environmental Science, Box 35, FI-40014 University of Jyväskylä, Finland

[c] University of Eastern Finland, Department of Environmental and Biological Sciences, P.O. Box 1627, 70211 Kuopio, Finland

*Corresponding author: petra.lindholm-lehto@luke.fi

Highlights

- Addition of peracetic acid decreased the levels of off-flavor compounds in water and fish.
- The decrease was observed on the levels of off-flavors, but not on the numbers of producers.
- *Streptomyces* was identified as the main GSM-producing species in recirculating water.

Abstract

In recirculating aquaculture systems (RAS)s, off-flavors and odors, mainly caused by geosmin (GSM) and 2-methylisoborneol (MIB), can accumulate in the flesh of fish from RAS water, reducing the profitability of production. In this study, peracetic acid (PAA) was applied in three application intervals to pump sumps of rainbow trout (*Oncorhynchus mykiss*) reared in RAS. Using a real-time polymerase chain reaction (qPCR), the potential off-flavor producers were quantified using *geo*A and MIB synthase genes. *Streptomyces* was identified as the major GSM producer, and biofilters showed the highest number of potential off-flavor producers. Concentrations of GSM and MIB were analyzed in the circulating water and in the lateral part of the fish fillet. In water, concentrations up to 51 ng L^{-1} (GSM) and 60.3 ng L^{-1} (MIB) were found, while in the fillet, these were up to 9.8 ng g^{-1} (GSM) and 10.2 ng g^{-1} (MIB), decreasing with increasing number of PAA applications. PAA applications reduced the levels of off-flavor compounds, although this was insufficient to fully prevent the accumulation of GSM and MIB.

Keywords: geoA; MIB synthase; off-flavors; rainbow trout; recirculating aquaculture system (RAS); qPCR

1. Introduction

Aquaculture is the fastest growing food-production sector and accounts for over 50 % of the world's fish consumption (Buric et al., 2014; FAO, 2017). Furthermore, land-based intensive recirculating aquaculture system (RAS) enables reduction in water consumption and nutrient discharge (Dalsgaard et al., 2013). Unfortunately, this has some drawbacks, such as a tendency for off-flavor compounds to

ACCEPTED MANUSCRIPT

develop in the circulating water and the flesh of fish, especially in salmonids (Schrader and Summerfelt, 2010; Burr et al., 2012). The rainbow trout is one of the most produced species (total production 106 thousand tons in 2010 in the Nordic countries, including RAS) reared in large-scale RAS (Dalsgaard et al., 2013).

Geosmin (GSM, *trans*-1,10-dimethyl-*trans*-9-decalol) and 2-methylisoborneol (MIB, (1-R-exo)-1,2,7,7-tetramethyl-bicyclo[2.2.1]heptan-2-ol) (Gerber, 1968, 1969) are the most common compounds causing off-flavor and odor problems in fish and seafood production (Auffret et al., 2011). Although these compounds are neither toxic nor associated with any serious health effects, they give rise to an unpleasant taste and odor in both water (Smith et al., 2002) and fish (Howgate, 2004). GSM and MIB have very low human detection limits and are quickly absorbed into lipid-rich tissues (Davidson et al., 2014). Even low amounts reduce fish quality, resulting in significant economic losses (Engle et al., 1995; Tucker, 2000).

GSM and MIB are produced by terpene synthases as secondary metabolites of a wide variety of microorganisms, such as actinomycetes, cyanobacteria, proteobacteria, and fungi (Dickschat et al., 2005; Ludwig et al., 2007). The enzymes and genes involved in biosynthesis were first discovered for *Streptomyces* (Cane and Watt, 2003; Cane et al., 2006), and later for cyanobacteria (Ludwig et al., 2007; Agger et al., 2008). The discovery of the biosynthetic mechanism has led to the development of several molecular detection methods targeting these genes for different producers (Ludwig et al., 2007; Auffret et al., 2011; Kutovaya and Watson, 2014; Lukassen et al., 2017). In RAS, a biofilter is generally the main reservoir of microbes in RAS (Leonard et al., 2002; Rurangwa and Verdegem, 2015), but GSM- and MIB-producing bacteria have also been found to colonize other RAS sections (Guttmann and van Rijn, 2008; Schrader and Summerfelt, 2010; Auffret et al., 2013). In particular, *Streptomyces*, actinomycetes, and myxobacteria are considered responsible for GSM and MIB production in RAS (Dickschat et al., 2005; Guttman and van Rijn, 2008; Schrader and Summerfelt, 2010). Recently, uncultured groups of GSM-producing bacteria have been detected by molecular methods in RAS (Lukassen et al., 2017). However, the development of long-term management solutions requires a more thorough understanding of the distribution and identity of GSM- and MIB-producing microorganisms in RAS.

Peracetic acid (PAA), a peroxide of acetic acid (CH_3CO_3H), is a commercially available strong antimicrobial disinfectant (Pedersen et al., 2009). PAA is effective in ambient conditions over a wide range of temperatures (Colgan and Gehr, 2001). It is stable, has a long shelf life, and comes at a reasonable cost (Luukkonen and Pehkonen, 2017), but there is a risk of explosion when it is highly concentrated, heated, or exposed to the catalytic effects of impurities (Wang et al., 2015a). In commercial products, PAA content ranges between 3-40 % and that of hydrogen peroxide between 14-35 %. PAA has a stronger antimicrobial effect than hydrogen peroxide (Alasri et al., 1992), but the combination of both has synergetic effects, and hydrogen peroxide is an additional source of hydroxyl radicals (Flores et al., 2014). The combination of PAA and hydrogen peroxide has bactericidal (at 0.001 %), fungicidal (at 0.003 %), sporicidal (at 0.3 %) and virucidal effects (at 0.75 %) (Greenspan and MacKellar, 1951; Baldry and French, 1989; Straus et al., 2012).

Unlike many other antimicrobial disinfectants, PAA degrades mainly through chemical oxidation into harmless acetic acid and water, producing non-toxic or mutagenic by-products (Monarca et al., 2002). It has neither lethal effect on fish nor impairs nitrification in biofilters at low dosages (1 mg L^{-1}) (Pedersen et al., 2009). In Europe, PAA has been approved for treating pathogens in aquaculture (Straus et al., 2012; Hushangi and Shekarabi, 2018). However, PAA has shown contradictory results: in some cases it prevents some troublesome parasites, while in others it has remained ineffective (Pedersen and Henriksen, 2011; Pedersen et al., 2013). Some studies (e.g. Meinelt et al., 2007a,

ACCEPTED MANUSCRIPT

2007b) evaluated the effect of a PAA application, but according to Pedersen et al. (2009), the effect of PAA on nitrification bacteria has not been reported. However, PAA has not been shown to selectively reduce GSM- and MIB-producing microorganisms. The success of PAA depends on environmental conditions, product formulation and stability, the applied dose, organic matter content, and decay in the aquaculture system (Pedersen et al., 2009). In addition to PAA, other oxidizing treatments have been studied which decrease off-flavor-induced problems in RAS. For example, ozone can be used for the treatment of incoming and, effluent water, or for controlling circulating water (Powell and Scolding, 2016). Ozone and hydrogen peroxide (H_2O_2) have been used successfully to remove GSM and MIB, but only from drinking water (Rurangwa and Verdegem, 2015). A variety of AOP treatments, such as ozone/H_2O_2, ozone/UV, and UV/H_2O_2 (Rosenfeldt, et al., 2005; Klausen and Grønborg, 2010; Rurangwa and Verdegem, 2015), can oxidize off-flavor compounds, but until now AOPs have not been widely tested in RAS. Methods based on photocatalysis (Fotiou et al., 2015; Xue et al., 2016) have shown promising results and may be an option in the future. However, depuration with clean water remains the only efficient method available thus far to remove off-flavors. Typically, large amounts of clean water are required for depuration. This is costly, and the fish can reduce in size, because they are not fed during the depuration period. Even a reduction in the depuration time due to the application of PAA would make fish production more cost-effective.

To date, a limited number of studies have investigated the effect of PAA on water quality and fish health on RAS farms (Pedersen et al., 2009; Pedersen et al., 2013). Goals of PAA applications have included the control of parasite infections, improved fish growth, fish health, and a variety of water quality parameters, but the effect on concentrations of GSM and MIB in fish flesh and in the circulating water has been less important. This research aimed to study the effects of PAA application on the GSM and MIB concentrations in rainbow trout fillet and in the circulating water, as well as on the microbial populations causing the formation of off-flavors in RAS. Additionally, the distribution and quantification of different GSM- and MIB-producing genes were included.

2 Materials and methods

2.1 Experimental setup and peracetic acid treatment

Three PAA application levels (1, 2, or 4 times per week) and a control tank without PAA were applied in 8 individual RAS, using randomly allocated duplicate systems for each treatment. A more detailed description of the experimental RAS facility is reported by Pulkkinen et al. (2018). In brief, each RAS consisted of a 500 L fish tank, separate water treatment and water quality control systems with total water volume of 760 L. The solids removal system included a waste feed collector, swirl separator, and drum filter with a mesh size of 60 µm. In the present trial, we used an up-flow fixed-bed bioreactor (150 L) filled with 80 L of RK Bioelemets heavy (750 m^2 m^{-3}) carrier material, which was stabilized to full maturity before start of the trial. Dissolved carbon dioxide was removed from the water using a forced-ventilated cascade aeration column with Bio-Blok 200 (EXPO-NET Danmark A/S, Denmark) filter media.

Oxygen levels in the fish tanks were maintained at 7.6-8.2 mg L^{-1} by injecting oxygen into the pump sumps and were monitored constantly. Water temperature was maintained at 15.4 ± 0.8 °C, and the water pH in the pump sump was maintained at 7.2 throughout the experiment. The pH was adjusted by adding a 20 % NaOH (aq) solution. Nitrite-N levels were kept below 0.2 mg L^{-1} and nitrate-N between 50 and 75 mg L^{-1}. All measured values were monitored constantly and adjusted as required. Surface water from an oligotrophic Lake Peurunka (area 694 ha, 59613 m^3) was used as replacement water at a relative renewal rate of 500 L kg^{-1} feed. Inlet water was filtered with an activated carbon

ACCEPTED MANUSCRIPT

filter and disinfected with UV light. The circulating water flow rate was set to 0.2 L s^{-1}, and the pump output to 60 % throughout the experiment.

PAA was applied to the pump sumps 1, 2, or 4 times per week, in quantities of 2.2 mg PAA L^{-1} per day. The application was conducted by adding 4 mL of PAA solution twice a day. The daily dose was selected based on the article of Pedersen et al. (2013), and by observing fish behavior during initial pretrial tests. Occurrence of any residual peroxide was tested with colorimetric peroxide test strips (MQuant™) twice a week during the first two weeks to ensure that residual peroxide did not remain in the system.

2.2 Fish handling

At the start of the study, there were 50 fish in each tank, weighing on average 130 ± 5 g. Each tank had a biomass of approximately 6500 g. At the end of the experiment, there were about 33 fish in each tank, weighing 411 ± 43 g with a biomass of about 13300 g. The fish were fed with BioMar Orbit 4.5 mm pellets (Denmark). During the experiment, the corresponding feeding ratio decreased from 1.5 % to 1.1 %. There were two intermediate weighings, the first at three weeks and the second at five weeks, to adjust feeding according to the correct tank biomass. Feed was given 10-12 times per day and light was provided 24 hours per day. The fish were visually inspected on a daily basis and any mortalities were removed and recorded. Additionally, any changes in fish behavior and observations during the experiment, gutting, and filleting were recorded.

2.3 Sampling, pretreatment and analysis

2.3.1 Sampling of fish and circulating water

Circulating water was sampled after 8 weeks and again after 13 weeks of treatment. The circulating water was taken from the tank water outlet flow, and from the pump sump. For chemical analysis, water samples were collected in 250 mL high-density polyethylene (HDPE) plastic jars with HDPE plastic caps and stored at -22 °C before the analysis. For molecular analysis, two replicate water samples from each sampling point were filtered through 0.22 µm filters (Express Plus® Membrane, 25 mm, GPWP) and the filters were frozen to -20 °C before DNA extraction.

To simulate the accumulation of bacterial biomass and especially GSM and MIB producers in the system, HDPE tank material pieces were placed in the tank and pieces of BIO-BLOK® 200 material in the aeration tower. After 8 and 13 weeks of treatment, replicate pieces of these biofilm samples were collected from the tank and aeration tower. Seven RK Bioelement carriers from each biofilter unit were also collected. Prior to DNA extraction, all the biofilm samples were frozen at -20 °C for a minimum of 24 hours. All the biofilm samples were sonicated for 2+2 min (Branson 1510) and freeze-dried (Alpha 1-4 D plus, Martin Christ Gefriertrocknungsanlagen GmbH).

After 13 weeks of the experiment, five fish were taken from each tank, euthanized, gutted, and frozen. The fish were later defrosted, filleted, and lateral part of the fillet, as defined by Hathurusingha and Davey (2016), were collected for analysis.

2.3.2 Pretreatment

Pretreatment for chemical analysis was performed as described by Lindholm-Lehto et al. (2019). In short, GSM and MIB were extracted from the sample matrix using HS-SPME. A volume of 1 mL of circulating water or 1 g of fish flesh was placed in a 10 mL HS vial, and 750 µL of saturated NaCl (aq) solution was added. A standard addition method was used to construct calibration curves for the quantification of GSM and MIB. Sealed sample vials were placed in a water bath at 60 °C. A

ACCEPTED MANUSCRIPT

DVB/CAR/PDMS fiber was exposed in the headspace and kept for 30 minutes to complete the SPME extraction before introducing the fiber directly into the GC-MS.

2.3.3 Chemical analysis

Peracetic acid (Bonsoxo 2901, Batch NO44817198), containing 12-13 w-% of peracetic acid and 19-23 w-% of hydrogen peroxide, was purchased from Bang & Bonsomer. NaOH (50 % solution) was purchased from Kemira Chemicals. Colorimetric peroxide test strips (0.5-25 mg L^{-1} H_2O_2, MQuant™) were purchased from Merck.

A standard solution (TraceCERT®, 100 µg mL^{-1} in MeOH) of (+/-) geosmin (GSM) and 2-methylisoborneol (MIB) was purchased from Merck. High-performance liquid chromatography (HPLC) grade methanol and hexane were obtained from J.T Baker and solid NaCl (purity 98 %) from Merck. Ultra-high quality (UHQ) water from Millipore (Bedford, MA, USA) was used in the analyses. Additionally, 10 mL headspace (HS) glass vials and polytetrafluoroethylene (PTFE) septum caps were purchased from Merck.

A manual solid phase micro extraction (SPME) assembly with a 1 cm, 50/30 µm extraction fiber (part no. 57328-U), coated with StableFlex divinylbenzene/ carboxene/ polydimethyl siloxane (DVB/CAR/PDMS), in a manual holder was purchased from Supelco (Merck).

Separation and quantification of GSM and MIB in the circulating water and in rainbow trout flesh were conducted using gas chromatography-mass spectrometry (GC-MS) equipment by Agilent 6890 series/5973 N GC/MSD (Palo Alto, CA, USA) system with a Phenomenex Zebron ZB-5MSi (Torrance, CA, USA) capillary column (30 m x 0.25 mm x 0.25 µm). The temperature of the injector was adjusted to 270 °C in the splitless mode. The carrier gas was helium at a flow rate of 0.7 mL min^{-1}. The temperature of the oven started at 45 °C for 3 min. and increased by 30 °C min^{-1} to achieve 300 °C (total time 14.5 min). The electron impact (EI)-MS conditions were selected as 230 °C for the ion source with a 5 min delay and ionizing voltage of 70 eV. The selected ion monitoring (SIM) mode was used for the detection of GSM and MIB with *m/z* 112, 126, 182 (GSM) and *m/z* 95, 135, 168 (MIB). Base peak areas of *m/z* 95 and *m/z* 112 were used for the quantification of GSM and MIB. A fully detailed method validation has been reported by Lindholm-Lehto et al. (2019).

2.3.4 Molecular analysis

Genomic DNA was extracted from freeze-dried biofilms and filters using the DNeasy PowerLyzer™ PowerSoil DNA Isolation Kit (Qiagen) in accordance with the manufacturer's instructions. The PowerLyzer Homogeniser was applied once at 3400 rpm for 45 s during the extraction. The amount of extracted DNA was measured with a Qubit 2.0 Fluorometer (Thermo Fischer Scientific). The abundance of partial 16S rRNA, *geo*A and MIB synthase genes was quantified with real-time quantitative PCR (qPCR). The 16S rRNA gene was amplified with 515F-Y (Parada et al., 2016) and 806r (Caporaso et al., 2011) primers according to protocol (Parada et al., 2016). For potential geosmin GSM producers, specific primers for *Streptomyces* (AMgeoF/R; Auffret et al., 2011), myxobacteria (*geo*A_g3F/R; Lukassen et al., 2017), *Sorangium* (*geo*A_g4F/R; Lukassen et al., 2017) and cyanobacteria (SGF1/JDR1; Tsao et al., 2014) producers were applied to the filter and biofilm samples. For the potential producers of MIB, primer pairs for cyanobacteria (Mtcf/r; Wang et al., 2011) and *Streptomyces* (Str-F/R; Wang et al., 2015b) were used.

The qPCR reactions included 6 ng of template DNA, 350 nM of both primers, and 1x Maxima SYBR Green/Fluorescein Master Mix (Thermo Fisher) in a total volume of 25 µl. For each sample, three replicate amplifications were used. The qPCR conditions were as follows: The initial denaturation step was at 95 °C for 10 min, with 35 cycles of 95 °C for 30 s, 52-65 °C for 30 s, and finally 72 °C for

ACCEPTED MANUSCRIPT

30 s. A melting curve analysis was performed in 0.5 °C increments for 5 s from 65 °C to 95 °C. The thermal conditions were similar for all genes, although the annealing temperature varied and was 52 °C for 16S rRNA, 55 °C for *Sorangium* and myxobacteria *geo*A, 58 °C for cyanobacterial *geo*A and MIB synthase, and 66 °C for *Streptomyces geo*A. The qPCR assays were performed with CFX96™ Real-Time PCR Detection System (Bio-Rad Laboratories) and analyzed with a CFX™ Manager (Bio-Rad). Standard curves were prepared either from genomic DNA of GSM-producing *Streptomyces griseus* DSM41217 and GSM- and MIB-producing cyanobacteria *Planktothrix* sp. UHCC328. Genomic DNA was used for *Streptomyces* and cyanobacterial assays, while standard curves for other assays were constructed from the amplification products. Amplicons from the qPCR run were extracted from the agarose gel with GenElute™ from several samples and pooled. 10-fold dilution series from 10^7 to 10^1 gene copies were used in each assay to calculate the gene copy numbers. Amplification efficiencies were determined from the regression slope of standard dilution series. The amplification efficiencies ranged between 89-100 %.

2.4 Statistical analyses

A regression analysis was conducted with SPSS Statistics software, version 25. Linear regression relationships between different PAA applications and GSM, MIB, and *geo*A were studied in samples after two sampling periods. The significance level was set at 0.05.

3 Results

3.1 GSM and MIB in RAS water

According to the results, concentrations ranged from below the level of detection (< LOD) to 48.7 ng L^{-1} for GSM (Fig. 1), and between < LOD and 41.4 ng L^{-1} for MIB (Fig. 2) after 8 weeks of the experiment. In the control and the systems with 1 PAA application per week, MIB levels increased after 13 weeks compared with 8 weeks, whereas for GSM, they increased in the control and systems with 2 and 4 PAA applications per week. After 8 weeks of the experiment, only GSM levels below 5 ng L^{-1} were detected with 4 PAA applications per week.

Fig. 1. Concentrations of GSM (ng L^{-1}) in RAS rearing rainbow trout. Water from the tank and pump sump, control and application of PAA 1, 2, or 4 times per week (2.2 mg PAA L^{-1} day^{-1}) for 8 and 13 weeks, n=8 (± SD).

After 13 weeks of the experiment, GSM and MIB levels had increased compared with those detected after 8 weeks (Fig. 1; Fig. 2), ranging between < LOD and 51.0 ng L^{-1} (GSM), and < LOD and 60.3 ng L^{-1} (MIB), respectively. Concentrations decreased slightly after 1 and 2 applications of PAA per week and even more after 4 applications per week. However, average concentrations in the tank water remained below 5 ng L^{-1} and in the pump sump at 10 ng L^{-1} for GSM and below 5 ng L^{-1} for MIB.

Fig. 2. Concentrations of MIB (ng L^{-1}) in RAS rearing rainbow trout. Water from the tank and pump sump, control and application of PAA 1, 2, or 4 times per week (2.2 mg PAA L^{-1} day^{-1}) for 8 and 13 weeks, n=8 (± SD).

The coefficient for correlating PAA applications per week and GSM and MIB concentrations resulted in R^2 values of 0.78 and 0.86 for GSM and 0.86 and 0.89 for MIB with 8 replicates in the tanks and pump sumps after 8 weeks. After 13 weeks, the correlations showed R^2 values of 0.52 and 0.80 for

ACCEPTED MANUSCRIPT

GSM, and 0.86 and 0.94 for MIB in the tanks and pump sumps. A linear regression equations, *p* values, and R^2 values were listed in Table 1. The values were listed only if there were significant correlations ($p < 0.05$).

Table 1. Linear regression relationships between different PAA applications with GSM, MIB and *geo*A in samples after 8 and 13 weeks. The significance level was set at 0.05.

Sample	Week	Equation	R^2	*p* value
*Streptomyces geo*A fish tank	8			ns
*Streptomyces geo*A pump sump	8			ns
*geo*A genes fish tank	8			ns
*geo*A genes aeration tower	8			ns
*geo*A genes bioreactor	8			ns
GSM fish tank	8	y=-0.082x+3.738	0.78	<0.01
GSM pump sump	8	y=-0.072x+3.521	0.86	<0.001
MIB fish tank	8	y=-0.115x+4.091	0.86	<0.001
MIB pump sump	8	y=-0.099x+3.908	0.89	<0.001
*Streptomyces geo*A fish tank	13			ns
*Streptomyces geo*A pump sump	13			ns
*geo*A genes fish tank	13			ns
*geo*A genes aeration tower	13			ns
*geo*A genes bioreactor	13			ns
GSM fish tank	13	y=-0.056x+3.357	0.52	0.026
GSM pump sump	13	y=-0.072x+3.559	0.80	<0.01
MIB fish tank	13	y=-0.067x+3.629	0.86	<0.001
MIB pump sump	13	y=-0.083x+3.769	0.94	<0.001
GSM fish	13	y=-0.250x+3.323	0.55	0.035
MIB fish	13	y=-0.383x+3.889	0.63	0.019

ns - not significant

3.2 Rainbow trout

3.2.1 GSM and MIB in fillet

In the studied rainbow trout fillets, concentrations ranged between < LOD and 9.8 ng g^{-1} for GSM, and between <LOD and 10.2 ng g^{-1} for MIB (Fig. 3), both decreasing with increasing weekly applications of PAA (Fig. 3). However, variations between replicates were relatively high. In the case of 2 applications per week, high GSM and MIB concentrations were measured in rainbow trout fillets from tank 5, increasing average concentrations (Fig. 3).

Concentrations in the fillets showed moderate correlations, with an R^2 value of 0.55 for GSM, and 0.63 for MIB. There were larger concentrations and variations especially in tank 5 (2 PAA applications per week), compared with the other tanks, reducing the R^2 values. However, the results of the weekly PAA applications and GSM and MIB concentrations gave $p < 0.05$.

3.2.2 Observations

The mortality rate ranged between 4-28 %, showing an increase after 4 weekly PAA applications. In the control tanks and after 1 and 2 PAA applications, the rate was between 4-22 %. However, the rate ranged between 11-28 % after four weekly PAA applications.

ACCEPTED MANUSCRIPT

Generally, mucus or slime on fish skin contains gel-forming macromolecules, which are glycoproteins excreted by the goblet cells of the fish skin (Fletcher et al., 1976). In this study, the fish exposed to PAA had developed an unusually thick layer of mucus and slime on the skin surface. The fish also showed unusual behavior and swam on the surface of the tank after PAA application, indicating stress or discomfort.

Fig. 3. Concentrations of GSM and MIB (ng g^{-1}) in RAS rearing rainbow trout. Samples from lateral part of rainbow trout fillet, control and application of PAA 1, 2, or 4 times per week (2.2 mg PAA L^{-1} day^{-1}) for 13 weeks, n=8 (± SD).

3.3 *geo*A genes in biofilms and recirculating water

*Geo*A gene copy numbers increased with PAA applications. Generally, the lowest number was determined from the control samples, except in the tank samples after 8 weeks, where the lowest number was found with four PAA applications. In the biofilm samples, the highest number of *geo*A genes was quantified from the biofilter after 8 and 13 weeks of the experiment (Fig. 4) in all RAS units. The second most common reservoir for *geo*A genes was the tank biofilm, where detected R^2=0.63 copy numbers were the lowest in systems with one PAA application ($2.3 \cdot 10^2$ cm^{-2}), and the highest in the systems with four PAA applications ($3.1 \cdot 10^4$ cm^{-2}). G*eo*A gene levels in tank 5 were considerably higher than those in the other tanks and in the biofilters. *Streptomyces* was identified as the main producer of GSM, while neither cyanobacterial nor *Sorangium* GSM was detected. In the recirculating water, only *Streptomyces* was detected according to qPCR. G*eo*A gene levels (*Streptomyces*) were generally higher in the pump sumps than in the tank water.

Fig. 4. The abundance of *geo*A $cm^{2\ -1}$ genes in the tank, aeration tower and biofilter biofilm (lines, secondary axis) with the control and application of PAA 1, 2, or 4 times per week (2.2 mg PAA L^{-1} day^{-1}) for 8 and 13 weeks, n= 8 (± SD).

The lowest number of *geoA* gene copies (340 mL^{-1}) in the circulating water samples was observed in the reference tanks after 8 weeks (Fig. 5), and the highest (1400 mL^{-1}) in the tank with two PAA applications after 13 weeks. Levels of up to 10.2 ng g^{-1} of MIB in fish and 60.3 ng L^{-1} in the recirculating water were detected by the chemical method, but MIB synthase gene copies of cyanobacteria remained below the detection limit. *Streptomyces* MIB producers were also detected after the different PAA applications.

Fig. 5. *Streptomyces geo*A gene copy numbers mL^{-1} in tank water and pump sump, with the control and application of PAA 1, 2, or 4 times per week (2.2 mg PAA L^{-1} day^{-1}) for 8 and 13 weeks, n=8 (± SD).

4 Discussion

The results suggest that an increased PAA dosage (4 times per week) can reduce GSM and MIB levels. Generally, several factors affect the levels of off-flavors in RAS, such as water temperature, fish density, water renewal rate, and the off-flavor concentration in the depuration system supply water (Dionigi et al., 2000; Howgate, 2004; Drake et al., 2010). Although PAA applications were seen to temporarily inhibit the production of GSM and MIB producers and to reduce GSM and MIB concentrations, the applied levels were inadequate to fully prevent GSM and MIB formation. Overall GSM and MIB concentrations in the circulating water were in the same range reported by Sarker et al.

ACCEPTED MANUSCRIPT

(2014). They reported 16 ng L^{-1} (GSM) in tank water and 30 ng L^{-1} in a biofilter. Somewhat higher concentrations of 161 ng L^{-1} (GSM) and 150 ng L^{-1} (MIB) were detected in the circulating water of a commercial barramundi (*Lates calcarifer*) RAS farm (Hathurusingha and Davey, 2016). Burr et al. (2012) found MIB concentrations between 50 and 130 ng L^{-1}, while Guttman and van Rijn (2008) reported levels of 170 ng L^{-1} (GSM) and 75 ng L^{-1} (MIB) in the circulating water of a tilapia (*Oreochromis niloticus* × *Oreochromis aureus*) farm. The detected levels of this study are in a similar range compared with those previously reported, and somewhat lower after PAA applications.

Detected concentrations in rainbow trout fillets were fairly high compared with previously reported results. For example, 0.094 ng g^{-1} (MIB) and 0.198 ng g^{-1} (GSM) were found in RAS farmed Atlantic salmon (*Salmo salar*) (Burr et al., 2012), while somewhat higher levels (0.2-0.9 ng g^{-1} for MIB and 0.10-0.115 ng g^{-1} for GSM) have also been found in Atlantic salmon (Davidson et al., 2014). However, concentrations of 0.27-0.59 ng g^{-1} (GSM) and 4.8-19.7 ng g^{-1} (MIB) have been found in RAS farmed rainbow trout (*Oncorhynchus mykiss*), which was in the same range as the results of this study (Zimba et al., 2012). Similarly, the results were close to those Sarker et al. (2014) detected, with 6 ng g^{-1} levels of GSM in rainbow trout fillets.

GSM has been found difficult to degrade with microbial reactions (Saito et al., 1999). Degradation reactions are largely caused by hydroxyl radical, while direct photolysis reactions play a minor role (Jo et al., 2011). Klausen and Grønborg (2010) showed that AOP treatments with UV/H_2O_2 and UV/O_3 could degrade GSM and MIB in RAS, but the degradation rate was reduced due to competitive reactions with organic matter. Klausen and Grønborg (2010) suggested a pretreatment before the AOP treatment to improve its feasibility. The disinfectant capability of PAA is a result of the release of active oxygen (Liberti and Notarnicola, 1999), while hydroxyl radicals formed during the homolysis of PAA are responsible for degradation reactions (Rokhina et al., 2010). Therefore, the reduction of concentrations in this study may not be due strictly to the inhibitive effects of PAA, but also to other reactions, such as GSM and MIB degradation.

Large variations between replicate tanks were observed during this study. Previous studies have revealed that individual tanks can differ substantially, especially in GSM concentrations (Guttman and van Rijn, 2008; Petersen et al., 2011). In this study, the biofilm materials in the aerobic compartments of the RAS were the main hosts for GSM- and MIB-producing bacteria, and most likely, MIB and GSM were released from the biofilms into the water. In this study, biofilter *geo*A gene copy numbers increased between weeks 8 and 13 of the experiment, an indication of the bacterial population established in the biofilter. The number of potential producers in an RAS system may change rapidly and differ greatly in the water and biofilter (Auffret et al., 2011).

The variation in GSM concentrations was higher in systems with two PAA applications per week compared with the other treatments. Two tanks were randomly selected for each application (control, 1, 2, or 4 applications per week). Although one of the two replicate tanks had 2 applications per week, it had unusually large numbers of *geo*A genes and high concentrations of GSM. The same tank also had systematically higher *geo*A gene copy numbers in other biofilm samples (Bio-Blok and the tank biofilm). The microbial total based on the 16S rRNA gene was congruent with other tanks. The percentage of potential GSM producers was thus higher in divergent tank. It is possible that the RAS units had been insufficiently cleaned before the start of the trial. Previous experiments may have affected the results, because there was an unusually high general specificity of the selected primers. However, in this study, myxobacteria primer pair *geo*A_g3F/R also produced positive results for *Streptomyces griseus*. This is because the lack of a Taqman-probe, and thus a primer pair, may have exaggerated the potential myxobacteria producers of this study.

ACCEPTED MANUSCRIPT

Molecular ecological methods targeting biosynthetic genes are widely used to quantify the potential producers of secondary metabolites. In this study, the quantity of *geo*A genes increased systematically from the control to the two PAA applications, and then decreased in systems with four PAA applications, except in the tank water after 13 weeks. Although the gene copy number rose with PAA applications, the GSM concentration in the water and fish decreased, indicating that the PAA had inactivated some metabolic pathways in GSM- and MIB-producing bacteria. PAA may inhibit the growth of other microbes in the system. Additionally, adaptation may favor bacteria which metabolize PAA, reducing the effect of PAA applications.

The water and biofilm in RAS host different bacterial communities (Rud et al., 2017). They can thus also host different GSM and MIB producers. Four PAA applications per week reduced the amount of potential GSM producing actinobacteria in different biofilms. Interestingly, previous studies have found that *Sorangium* and myxobacteria or *Nannocystis* are responsible for GSM production (Auffret et al., 2013; Lukassen et al., 2017). The most abundant producer of GSM in this study was *Streptomyces*. This suggests that different freshwater RAS systems host very different producers of the odorous metabolites GSM and MIB.

Streptomyces MIB producers were also detected from tank and pump sump water samples during the experiment (Supplementary Table 1). However, an additional band appeared in the agarose gel run in some reactions performed with a MIBStr primer pair, meaning reliable gene copy quantification was impossible. The presence of MIB *Streptomyces* genes in the system explains the measured MIB concentrations, although it is probable that the primer pairs were suboptimal for the detection of all the possible MIB producers.

High *geo*A gene levels seem to indicate high levels of GSM. However, high levels of MIB were found in a tank with 2 PAA applications per week, despite the fact that no genes associated with MIB formation could be quantified. In general PCR, some tanks produced positive results for MIB synthase genes. Detection methods based on PCR and qPCR for potential MIB producers are limited to only a few genera, while a much wider range of methods exists for detecting GSM producers (e.g. Auffret et al., 2011; Kutovaya and Watson, 2014; Suurnäkki et al., 2015). Although there are several methods for detecting MIB synthase genes (Wang et al., 2011; Suurnäkki et al., 2015; Chiu et al., 2016), they have not been applied to RAS systems. Direct molecular methods largely depend on the available sequence information. MIB producers in this study and RAS systems in general may harbor yet unknown types of MIB synthase, and current methods may therefore underestimate their abundances.

Fish skin is influenced by factors related to the season, developmental stage, environmental conditions, stress, and disease (Shepard, 1994). PAA-induced changes in the circulating water may therefore have caused excess mucus formation. Chronic stress may lead to increased compensatory metabolic activity in fish, resulting in reduced growth and suppressed immunity (Liu et al., 2018). Fish adapt to PAA-induced stress quickly, but with minor prolonged increases in cortisol level. For example, low PAA dosages in RAS culture water have led to increased stress hormone cortisol levels in mirror carp, *Cyprinus carpio* (Liu et al., 2018). We did not assess immunity or other physiological indicators in this study. However, it seems that PAA application has led to increased mucus formation on fish skin and temporary changes in fish behavior.

5 Conclusions

The application of increasing PAA dosages reduced GSM and MIB levels in the circulating water and in the rainbow trout fillets. However, the effect remained only temporary, and concentrations gradually increased during the experiment period. The highest numbers of *geo*A genes were quantified from

ACCEPTED MANUSCRIPT

the biofilter in all RAS units, *Streptomyces* being the main GSM producer. Although the gene copy numbers increased with the increasing PAA dosage, the GSM concentration in the water and fish was reduced, indicating that PAA treatment was able to deactivate some metabolic pathways in GSM- and MIB-producing bacteria. However, the MIB synthase gene of actinobacteria and cyanobacteria remained undetected, despite the fact that MIB concentrations were in the same range as those of GSM. In this study, the PAA dose was in the upper safe dosage range in terms of fish welfare, but it was still unable to fully prevent the formation of off-flavor-producing bacteria. In conclusion, PAA reduces the levels of off-flavor-producing compounds, but it is still insufficient to fully resolve off-flavor-induced challenges.

Acknowledgements

Financial support from the European Maritime and Fisheries Fund and the Academy of Finland is gratefully acknowledged.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Author contributions

The experiment was planned by Vielma, Pulkkinen, and Lindholm-Lehto. Lindholm-Lehto and Suurnäkki conducted the sample preparations and analyses. Lindholm-Lehto and Suurnäkki drafted the manuscript. Vielma, Tiirola, Aalto, and Pulkkinen critically examined and revised the manuscript.

References

Agger, S.A., Lopez-Gallego, F., Hoye, T.R., Schmidt-Dannert, C., 2008. Identification of sesquiterpene synthases from *Nostoc punctiforme* PCC 73102 and *Nostoc* sp. strain PCC 7120. J. Bacteriol. 190, 6084-6096. doi:10.1128/JB.00759-08.

Alasri, A., Roques, C., Michel, G., Cabassu, C., Aptel, P., 1992. Bactericidal properties of peracetic-acid and hydrogen-peroxide, alone and in combination, and chlorine and formaldehyde against bacterial wastewater. Can. J. Microbiol. 38, 635-642. doi.org/10.1139/m92-104.

Auffret, M., Pilote, A., Proulx, E., Proulx, D., Vandenberg, G., Villemur, R., 2011. Establishment of a real-time PCR method for quantification of geosmin-producing *Streptomyces* spp. in recirculating aquaculture systems. Water Res. 45, 6753-6762. doi:10.1016/j.watres.2011.10.020.

Auffret, M., Yergeau, E., Pilote, A., Proulx, É., Proulx, D., Greer, C. W., Vandenberg G., Villemur, R., 2013. Impact of water quality on the bacterial populations and off-flavours in recirculating aquaculture systems. FEMS Microbiol. Ecol. 84, 235-247. doi.org/10.1111/1574-6941.12053.

Baldry, M.G.C., French M.S., 1989. Activity of peracetic acid against sewage indicator organisms. Water Sci. Technol. 21, 1747-1749.

Buric, M., Bláhovec, J., Kouril, J., 2014. A simple and effective recirculating hatchery for salmonids. J. Aquacult. Res. Develop. 5, 271. doi:10.4172/2155-9546.1000271.

Burr, G.S., Wolters, W.R., Schrader, K.K., Summerfelt, S.T., 2012. Impact of depuration of earthy-musty off-flavors on fillet quality of Atlantic salmon, *Salmo salar*, cultured in a recirculating aquaculture system. Aquacult. Eng. 50, 28-36. doi:10.1016/j.aquaeng.2012.03.002.

Cane, D.E., Watt, R.M., 2003. Expression and mechanistic analysis of a germacradienol synthase from *Streptomyces coelicolor* implicated in geosmin biosynthesis. Proc. Nat. Acad. Sci. U.S.A. 100, 1547-1551. doi:10.1073/pnas.0337625100.

Cane, D.E., He, X., Kobayashi, S., Omura, S., Ikeda, H., 2006. Geosmin biosynthesis in *Stretomyces avermitilis*. Molecular cloning, expression, and mechanistic study of the germacradienol/geosmin synthase. J. Antibiot. 59, 471-479.

ACCEPTED MANUSCRIPT

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Frierer, N., Knight, R., 2011. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. Multidis. J. Microb. Ecol. 6, 1621-1624. doi:10.1038/ismej.2012.8.

Chiu, Y-T., Yen, H-K. Lin, T-F., 2016. An alternative method to quantify 2-MIB producing cyanobacteria in drinking water reservoir: method development and field applications. Environ. Res. 151, 618-627. doi: 10.1016/j.envres.2016.08.034.

Colgan, S., Gehr, R., 2001. Disinfection. Peracetic acid gains favor as an effective, environmentally benign disinfection alternative for municipal wastewater treatment applications. Water Environ. Technol. 13, 29-33.

Dalsgaard, J., Lund, I., Thorarinsdottir, R., Drengstig, A., Arvonen, K., Bovbjerg Pedersen, P., 2013. Farming different species in RAS in Nordic countries: Current status and future perspectives. Aquacult. Eng. 53, 2-13. doi:10.1016/j.aquaeng.2012.11.008.

Davidson, J., Schrader, K., Ruan, E., Swift, B., Aalhus, J., Juarez, M., Wolters, W., Burr, G., Good, C., Summerfelt, S.T., 2014. Evaluation of depuration procedures to mitigate the off-flavor compounds geosmin and 2-methylisoborneol from Atlantic salmon *Salmo salar* raised to market-size in recirculating aquaculture systems. Aquacult. Eng. 61, 27-34. doi.org/10.1016/j.aquaeng.2014.05.006.

Dickschat, J.S., Bode, H.B., Mahmud, T., Müller, R., Schulz. S., 2005. A novel type of geosmin biosynthesis in myxobacteria. J. Org. Chem. 70, 5174-5182. doi:10.1021/jo050449g.

Dionigi, C.P., Johnsen, P.B., Vinyard, B.T., 2000. The recovery of flavor quality by channel catfish. N. Am. J. Aquacult. 62, 189-194. doi.org/10.1577/1548-8454(2000)062<0189:TROFQB>2.3.CO;2.

Drake, S.L., Drake, M.A., Sanderson, R., Daniels, H.V., Yates, M.D., 2010. The effect of purging time on the sensory properties of aquacultured southern flounder (*Paralichthys lethostigma*). J. Sens. Stud. 25, 246-259. doi:10.1111/j.1745-459X.2009.00255.x.

Engle, C.R., Pounds, G. L., van der Ploeg, M., 1995. The cost of off-flavor. J. World Aquac. Soc. 26, 297-306. doi.org/10.1111/j.1749-7345.1995.tb00258.x.

FAO, 2017. Food and Agriculture Organization (FAO). Fisheries and Aquaculture Statistics; FAO Year Book; FAO: Rome, Italy.

Fletcher, T.C., Jones, R., Reid, L., 1976. Identification of glycoproteins in goblet cells of epidermis and gill of plaice (*Pleuroneces platessa L.*), flounder (*Platichthys flesus L.*) and rainbow trout (*Salmo gairdneri Richardson*). Histoc. J. 8, 597-608.

Flores, M.J., Lescano, M.R., Brandi, R.J., Cassano, A.E., Labas, M.D., 2014. A novel approach to explain the inactivation mechanism of *Escherichia coli* employing a commercially available peracetic acid. Water Sci. Technol. 69, 358-363. doi:10.2166/wst.2013.721.

Fotiou, T., Triantis, T.M., Kaloudis, T., Hiskia, A., 2015. Evaluation of the photocatalytic activity of TiO2 based catalysts for the degradation and mineralization of cyanobacterial toxins and water off-odor compounds under UV-A, solar and visible light. Chem. Eng. J. 261, 17-26. doi.org/10.1016/j.cej.2014.03.095.

Gerber, N.N., 1968. Geosmin from microorganisms in trans-1,10-dimethyl-trans-9-decalol. Tetrahed. Lett. 25, 2971-2974. doi:10.1016/S0040-4039(00)89625-2.

Gerber, N.N., 1969. A volatile metabolite of actinomycetes: 2-methylisoborneol. J. Antibiot. 22, 508-509. doi:10.7164/antibiotics.22.508.

Greenspan, F.P., MacKellar, D.G., 1951. The application of peracetic acid germicidal washes to mold control of tomatoes. Food Technol. 5, 95-97.

Guttman, L., van Rijn, J., 2008. Identification of conditions underlying production of geosmin and 2-methylisoborneol in a recirculating system. Aquaculture. 279, 85-91. doi:10.1016/j.aquaculture.2008.03.047.

Hathurusingha, P., Davey, K.R., 2016. Experimental validation of a time-dependent model for chemical taste taint accumulation as geosmin (GSM) and 2-methylisoborneol (MIB) in commercial RAS farmed barramundi (*Lates calcarifer*). Ecol. Model. 340, 17-27. doi:10.1016/j.ecolmodel.2016.08.017.

Howgate, P., 2004. Tainting of farmed fish by geosmin and 2-methyl-iso-borneol: a review of sensory aspects and of uptake/depuration. Aquaculture 234, 155-181. doi:10.1016/j.aquaculture.2003.09.032.

Hushangi, R., Shekarabi, S.P.H., 2018. Effect of a peracetic acid-based disinfectant on growth, hematology and histology of juvenile rainbow trout (*Oncorhynchus mykiss*). Fishes 3, 1-8. doi:10.3390/fishes3010010.

ACCEPTED MANUSCRIPT

Jo, C.H., Dietrich, A.M., Tanko, J.M., 2011. Simultaneous degradation of disinfection byproducts and earthy-musty odorants by the UV/H_2O_2 advanced oxidation process. Water Res. 45, 2507-2516. doi:10.1016/j.watres.2011.02.006.

Klausen, M.M., Grønborg, O., 2010. Pilot scale testing of advanced oxidation processes for degradation of geosmin and MIB in recirculated aquaculture. Water Sci. Technol.: Water Supply 10, 217-225. doi:10.2166/ws.2010.246.

Kutovaya, O.A., Watson, S.B., 2014. Development and application of a molecular assay to detect and monitor geosmin-producing cyanobacteria and actinomycetes in Great Lakes. J. Great Lakes Res. 40, 404-414. doi:10.1016/j.jglr.2014.03.016.

Leonard, N., Guiraud, J.P., Gasset, E., Cailleres, J.P., Blancheton, J.P., 2002. Bacteria and nutrients–nitrogen and carbon–in a recirculating system for sea bass production. Aquacult. Eng. 26, 111–127. doi:10.1016/S0144-8609(02)00008-0.

Liberti, L., Notarnicola, M.. 1999. Advanced treatment and disinfection for municipal wastewater reuse in agriculture. Water Sci. Technol. 40, 235-245. doi.org/10.2166/wst.1999.0596.

Lindholm-Lehto, P.C., Pakkanen, H., Vielma, J., Alén, R., (2019). Depuration of geosmin- and 2-methylisoborneol-induced off-flavors in recirculating aquaculture system farmed European whitefish *Coregonus lavaretus*. Aquacult. Res. (under review).

Liu, D., Straus, D.L., Pedersen, L.F., Meinelt, T., 2018. Periodic bacterial control with peracetic acid in a recirculating aquaculture system and its long-term beneficial effect on fish health. Aquaculture 485, 154-159. doi:10.1016/j.aquaculture.2017.11.050.

Ludwig, F., Medger, A., Börnick, H., Opitz, M., Lang, K., Göttfert, M., Röske, I., 2007. Identification and expression analyses of putative sesquiterpene synthase genes in *Phormidium sp*. and prevalence of *geo*A-like genes in a drinking water reservoir. Appl. Environ. Microbiol. 73, 6988-6993. doi:10.1128/AEM.01197-07.

Lukassen, M.B., Saunders, A.M., Sindilariu, P-D., Nielsen, J.L., 2017. Quantification of novel geosmin-producing bacteria in aquaculture systems. Aquaculture 479, 304-310. doi.org/10.1016/j.aquaculture.2017.06.004.

Luukkonen, T., Pehkonen, S.O., 2017. Peracids in water treatment: A critical review. Crit. Rev. Environ. Sci. Technol. 47, 1-39. doi.org/10.1080/10643389.2016.1272343.

Meinelt, T., Staaks, J., Staaks, G., Stueber, A., Braunig, I. (2007a). Anti-parasitic effects of peracetic acid (PAA) to free infective stages (Theronts) of the white spot disease, *Ichthyophthirius multifiliis in vitro*. Deutsche Tierarztliche Wochenschrift 114, 384-387.

Meinelt, T., Richert, I., Stuber, A., Braunig, I. (2007b). Application of peracetic acid to the parasite *Ichthyophthirius-multifiliis* in Sander (*Sander lucioperca*) breeding. Deutsche Tierarztliche Wochenschrift 114, 244-251.

Monarca, S., Richardson, S.D., Feretti, D., Grottolo, M., Thruston, A.D., Zani, C., Navazio, G., Ragazzo, P., Zerbini, I., Alberti, A., 2002. Mutagenicity and disinfection by-products in surface drinking water disinfected with peracetic acid. Environ. Toxicol. Chem. 21, 309-318. doi.org/10.1002/etc.5620210212.

Parada, A.E., Needham, D.M, Fihrman, J.A., 2016. Every base matters: assessing small subunit rRNA primer for marine microbiomes with mock communities, time series and global field samples. Environ. Microbiol. 18, 1403-1414. doi.org/10.1111/1462-2920.13023.

Pedersen, L.-F., Pedersen, P.B., Nielsen, J.L., Nielsen, P.H., 2009. Peracetic acid degradation and effects on nitrification in recirculating aquaculture systems. Aquaculture 296, 246-254. doi.org/10.1016/j.aquaculture.2009.08.021.

Pedersen, L.-F., Henriksen, N.H., 2011. Dambrugsteknologi: Formalin Substitution (in Danish). DTU Aqua Report 236.

Pedersen, L.-F., Meinelt, T., Straus, D.L., 2013. Peracetic acid degradation in freshwater aquaculture systems and possible practical implications. Aquacult. Eng. 53, 65-71. doi: 10.1016/j.aquaeng.2012.11.011.

Petersen, M.A., Hyldig, G., Strobel, B.W., Henriksen, N.H., Jørgensen, N.O.G., 2011. Chemical and sensory quantification of geosmin and 2-methylisoborneol in rainbow trout (*Oncorhynchus mykiss*) from recirculated aquacultures in relation to concentrations in basin water. J. Agricult. Food Chem. 59, 12561-12568. doi:10.1021/jf2033494.

ACCEPTED MANUSCRIPT

Powell, A., Scolding, J.W.S., 2016. Direct application of ozone in aquaculture systems. Rev. Aquacult. 0, 1-15. doi.org/10.1111/raq.12169.

Pulkkinen, J.T., Kiuru, T., Aalto, S.L., Koskela, J., Vielma, J., 2018. Startup and effects of relative water renewal rate on water quality and growth of rainbow trout (*Oncorhynchus mykiss*) in a unique RAS research platform. Aquacult. Eng. 82, 38-45. doi.org/10.1016/j.aquaeng.2018.06.003.

Rokhina, E.V., Makarova, K., Golovina, E.A., Van As, H., Virkutyte, J., 2010. Free radical reaction pathway, thermochemistry of peracetic acid homolysis, and its application for phenol degradation: Spectroscopic study and quantum chemistry calculations. Environ. Sci. Technol. 44, 6815-6821. doi: 10.1021/es1009136.

Rosenfeldt, E.J., Melcher, B., Linden, K.G., 2005. UV and UV/H_2O_2 treatment of methylisoborneol (MIB) and geosmin in water. J. Water Suppl. Res. Technol. AQUA, 54, 423-434.

Rud, I., Kolarevic, J., Holan, A.B., Berget, I., Calabrese, S., Terjesen, B.F., 2017. Deep-sequencing of the bacterial microbiota in commercial-scale recirculating and semi-closed aquaculture systems for Atlantic salmon post-smolt production. Aquacult. Eng. 78, 50-62. doi: 10.1016/j.aquaeng.2016.10.003.

Rurangwa, E., Verdegem, M.C.J., 2015. Microorganisms in recirculating aquaculture systems and their management. Rev. Aquacult. 7, 117-130. doi:10.1111/raq.12057.

Saito, A., Tokuyama, T., Tanaka, A., Oritani, T., Fuchigami, K., 1999. Microbiological degradation of (-)-geosmin. Water Res. 33, 3033-3036. doi.org/10.1016/S0043-1354(99)00155-4.

Sarker, P., Pilote, A., Auffret, M., Proulx, E., Villemur, R., Deschamps, M.-H., Vandenberg, G., 2014. Reducing geosmin off-flavor compounds and waste outputs through dietary phosphorus management in rainbow trout aquaculture. Aquacult. Environ. Interact. 6, 105-117. doi:10.3354/aei00119.

Schrader, K.K., Summerfelt, S.T., 2010. Distribution of off-flavor compounds and isolation of geosmin producing bacteria in a series of water recirculating systems for rainbow trout culture. N. Am. J. Aquacult. 72, 1-9. doi:10.1577/A09-009.1N.

Shepard, K.L., 1994. Functions for fish mucus. Rev. Fish Biol. Fisher. 4, 401-429. doi:10.1007/BF00042888.

Smith, V.H., Sieber-Denlinger, J., deNoyelles, F., Campbell, S., Pan, S., Randtke, S.J., Blain, G.T., Strasser, V.A., 2002. Managing taste and odor problems in a eutrophic drinking water reservoir. Lake Reserv. Manage. 18, 319-323. doi.org/10.1080/07438140209353938.

Straus, D.L., Meinelt, T., Farmer, B.D., Mitchell, A.J., 2012. Peracetic acid is effective for controlling fungus on channel catfish eggs. J. Fish Dise. 35, 505-511. doi.org/10.1111/j.1365-2761. 2012.01383.x.

Suurnäkki, S., Gomez-Saez, G.V., Rantala-Ylinen, A., Jokela, J., Fewer, D.P., Sivonen, K., 2015. Identification of geosmin and 2-methylisoborneol in cyanobacteria and molecular detection methods for the producers of these compounds. Water Res. 68, 56-66. doi.org/10.1016/j.watres.2014.09.037.

Tsao, H-W., Michinaka, A., Yen, H-K., Giglio, S., Hobson, P., Monis, P., Lin, T-F., 2014. Monitoring of geosmin producing *Anabaena circinalis* using quantitative PCR. Water Res. 49, 416-425. doi.org/10.1016/j.watres.2013.10.028.

Tucker, C.S., 2000. Off-flavor problems in aquaculture. Rev. Fish Sci. 8, 45-88. doi.org/10.1080/ 10641260091129170.

Wang, Z., Xu, Y., Shao, J., Wang, J., Li, R., 2011.Genes associated with 2-methylisoborneol biosynthesis in cyanobacteria: Isolation, characterization, and expression in response to light. PLoS One, 6, e18665. doi:10.1371/journal.pone.0018665.

Wang, Y.-W., Liao, M.-S., Shu, C.-M., 2015a. Thermal hazards of a green antimicrobial peracetic acid combining DSC calorimeter with thermal analysis equations. J. Thermal Anal. Calorim. 119, 2257-2267. doi:10.1007/s10973-014-4369-z.

Wang Z., Song G., Shao J., Tan W., Li Y., Li R., 2015b. Establishment and field applications of real-time PCR methods for the quantification of potential MIB-producing cyanobacteria in aquatic systems. J. Appl. Phycol. 28, 325-333. doi:10.1007/s10811-015-0529-1.

Xue, Q., Liu, Y., Zhou, Q., Utsumi, M., Zhang, Z., Sugiura, N., 2016. Photocatalytic degradation of geosmin by Pd nanoparticle modified WO3 catalyst under simulated solar light. Chem. Eng. J. 283, 614-621. doi.org/10.1016/j.cej.2015.08.016.

Zimba, P.V., Schrader, K.K., Hyldig, G., Strobel, B.W., Jørgensen, N.O.G., 2012. Evaluation of geosmin and 2-methylisoborneol off-flavour in smoked rainbow trout fillets using instrumental and sensory analyses. Aquacult. Res. 43, 149-153. doi:10.1111/j.1365-2109.2011.02795.x.

ACCEPTED MANUSCRIPT

Figures

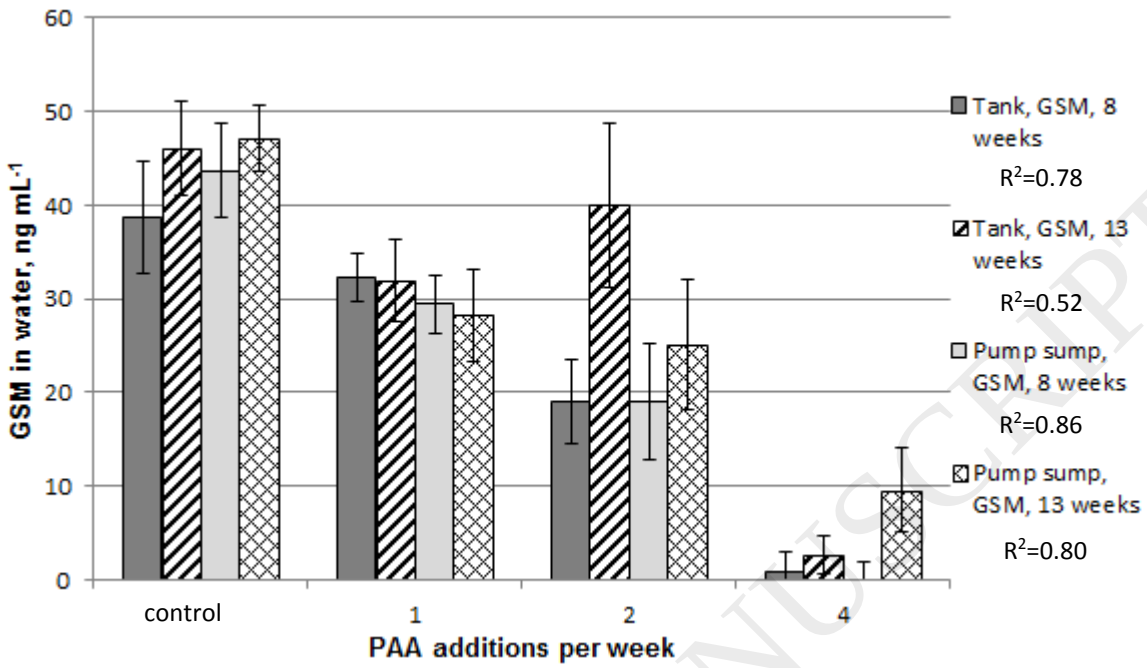


Fig. 1. Concentrations of GSM (ng L^{-1}) in RAS rearing rainbow trout. Water from the tank and pump sump, control and application of PAA 1, 2, or 4 times per week (2.2 mg PAA L^{-1} day^{-1}) for 8 and 13 weeks, n=8 (± SD).

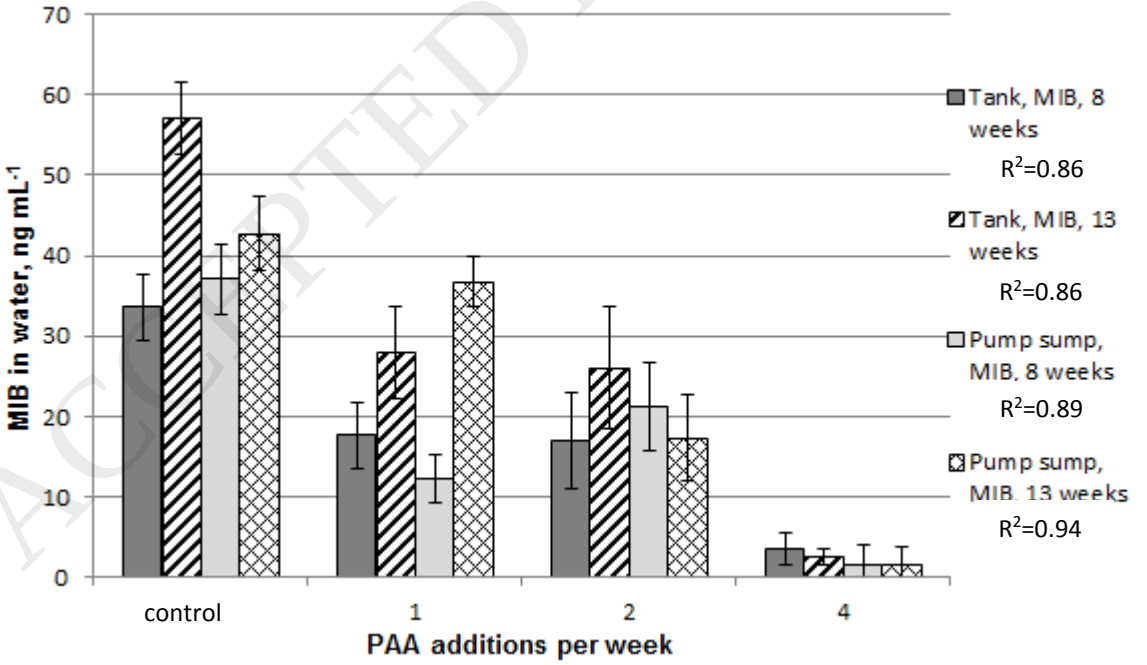


Fig. 2. Concentrations of MIB (ng L^{-1}) in RAS rearing rainbow trout. Water from the tank and pump sump, control and application of PAA 1, 2, or 4 times per week (2.2 mg PAA L^{-1} day^{-1}) for 8 and 13 weeks, n=8 (± SD).

ACCEPTED MANUSCRIPT

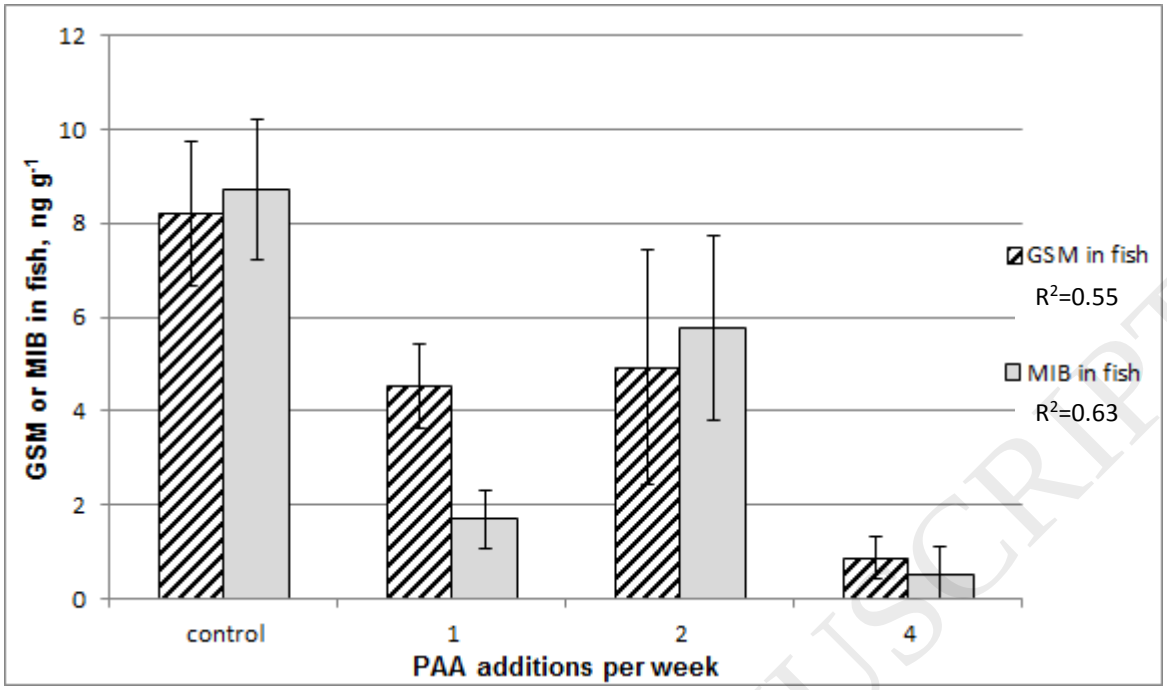


Fig. 3. Concentrations of GSM and MIB (ng g^{-1}) in RAS rearing rainbow trout. Samples from lateral part of rainbow trout fillet, control and application of PAA 1, 2, or 4 times per week (2.2 mg PAA L^{-1} day^{-1}) for 13 weeks, n=8 (± SD).

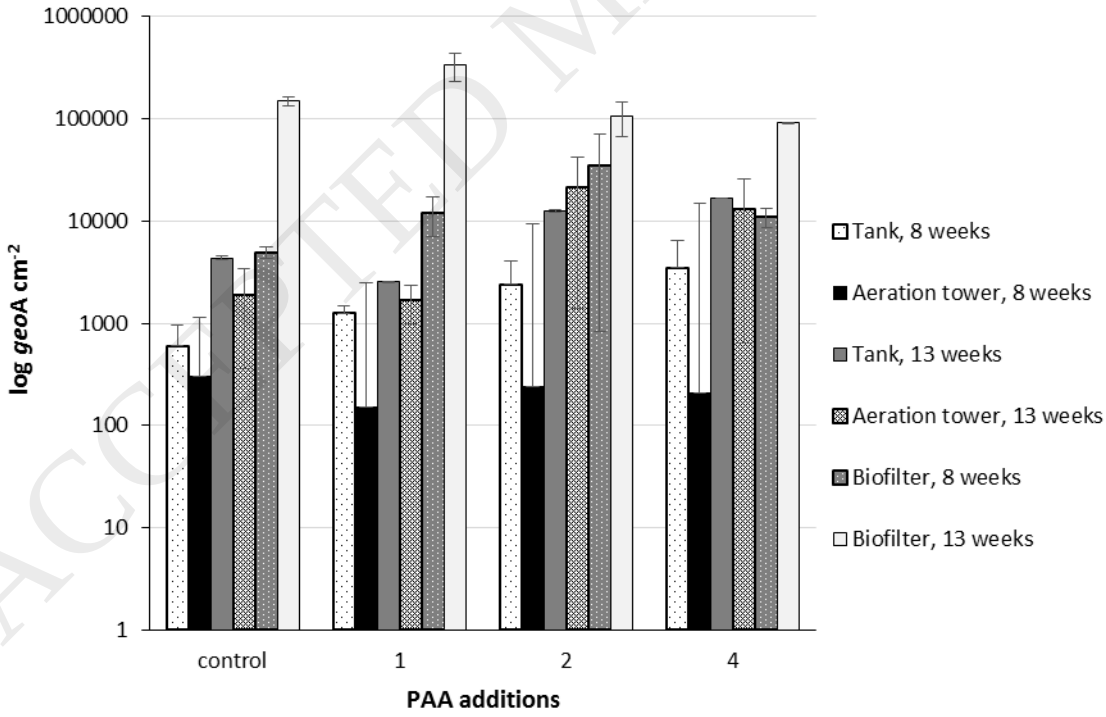


Fig. 4. The abundance of *geo*A $cm^{2\ -1}$ genes in the tank, aeration tower and biofilter biofilm (lines, secondary axis) with the control and application of PAA 1, 2, or 4 times per week (2.2 mg PAA L^{-1} day^{-1}) for 8 and 13 weeks, n= 8 (± SD).

ACCEPTED MANUSCRIPT

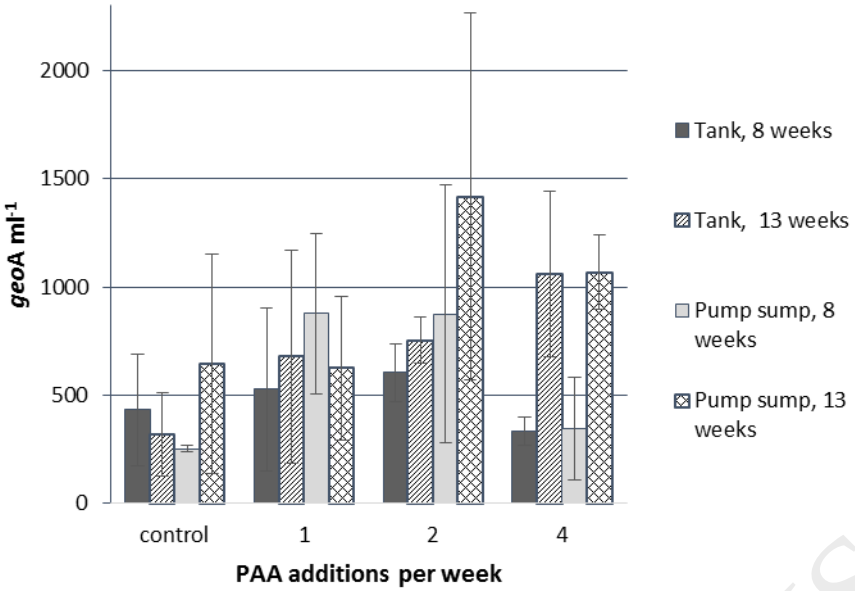


Fig. 5. *Streptomyces geo*A gene copy numbers mL^{-1} in tank water and pump sump, with the control and application of PAA 1, 2, or 4 times per week (2.2 mg PAA L^{-1} day^{-1}) for 8 and 13 weeks, n=8 (± SD).

ACCEPTED MANUSCRIPT